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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 24 Jun 2004 (20040624/PD)
FILE LAST UPDATED: 24 Jun 2004 (20040624/ED)
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HIGHEST APPLICATION PUBLICATION NUMBER: US2004123365
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=> e weiner david/in
E1            3     WEINER DANIEL/IN
E2            1     WEINER DANIEL L/IN
E3            9 --> WEINER DAVID/IN
E4            2     WEINER DAVID A/IN
E5           40     WEINER DAVID B/IN
E6            4     WEINER DAVID PAUL/IN
E7            4     WEINER DAVID WILLIAM/IN
E8            1     WEINER DONALD D/IN
E9            7     WEINER DOUGLAS B/IN
E10           5     WEINER DOUGLAS S/IN
E11           1     WEINER EDWARD G/IN
E12           1     WEINER ETHAN S/IN

=> s e5
L1           40 "WEINER DAVID B"/IN

=> d l1,ti,1-5
 L1   ANSWER 1 OF 40  USPATFULL on STN

TI     Dna vaccines encoding hiv accessory proteins

L1   ANSWER 2 OF 40  USPATFULL on STN
TI     Composition and methods of using hiv vpr

L1   ANSWER 3 OF 40  USPATFULL on STN
TI     Compositions for and methods of using herpes simplex virus glycoprotein
       d to suppress immune responses

L1   ANSWER 4 OF 40  USPATFULL on STN
TI     Compositions and methods for the abrogation of cellular proliferation
       utilizing the human immunodeficiency virus VPR protein

L1   ANSWER 5 OF 40  USPATFULL on STN
TI     VPR function and activity


=> s e3 or e5
             9 "WEINER DAVID"/IN
            40 "WEINER DAVID B"/IN
L2          49 "WEINER DAVID"/IN OR "WEINER DAVID B"/IN

=> s l2 and (Vpr or viral protein R)
          1091 VPR
         65975 VIRAL
        174530 PROTEIN
        977413 R
            32 VIRAL PROTEIN R
                 (VIRAL(W)PROTEIN(W)R)
L3          26 L2 AND (VPR OR VIRAL PROTEIN R)
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=> s l3 and antibod?
        103710 ANTIBOD?
L4          26 L3 AND ANTIBOD?

=> d l4,cbib,ab,clm,1-26

L4   ANSWER 1 OF 26  USPATFULL on STN
2004:138927 Dna vaccines encoding hiv accessory proteins.
    **Weiner, David B.**, Merion Station, PA, UNITED STATES
    Ayvavoo, Velpandi, Pittsburgh, PA, UNITED STATES
    US 2004106100 A1 20040603
    APPLICATION: US 2003-312197  A1  20030516 (10)
    WO 2001-US41357 20010712
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     Improved vaccines and methods of using the same are disclosed. Immunosuppressive compositions for treating individuals who have autoimmune diseases or transplants and methods of using the same are disclosed.
CLM    What is claimed is:
       1. A polyprotein comprising HIV Vif, HIV Vpu and HIV Nef.

       2. The polyprotein of claim 1 wherein HIV Vif is SEQ ID NO:1.

       3. The polyprotein of claim 1 wherein HIV Vpu is SEQ ID NO:2.

       4. The polyprotein of claim 1 wherein HIV Nef is SEQ ID NO:3.

       5. The polyprotein of claim 1 wherein HIV Vif, HIV Vpu and HIV Nef are present in the order HIV Vif, HV Vpu and HIV Nef relative to each other from N terminal to C terminal.

       6. The polyprotein of claim 5 wherein a protease cleavage site is located in between HIV Vif, and HIV Vpu and a protease cleavage site is located in between HIV Vpu and HIV Nef.

       7. The polyprotein of claim 6 wherein HIV Vif is SEQ ID NO:1.

       8. The polyprotein of claim 6 wherein HIV Vpu is SEQ ID NO:2.

       9. The polyprotein of claim 6 wherein HIV Nef is SEQ ID NO:3.

       10. The polyprotein of claim 6 wherein the protease cleavage site is REKRAVVG.

       11. The polyprotein of claim 1 wherein HIV Vif is SEQ ID NO:1; HIV Vpu is SEQ ID NO:2 and HIV Nef is SEQ ID NO:3.

       12. An nucleic acid molecule comprising a coding sequence encoding the polyprotein of claims 1-11.

       13. The nucleic acid molecule of claim 12 wherein said coding sequence operably linked to regulatory elements.

       14. The nucleic acid molecule of claim 12 wherein said nucleic acid molecule is a plasmid

       15. A plasmid of claim 14 wherein said coding sequence is operably linked to regulatory elements.

       16. A recombinant vaccine or attenuated vaccine comprising a nucleic acid molecule of claim 12.

       17. A pharmaceutical composition comprising the subject matter of claims 1-16.

18. A methods immunizing and individual against HIV comprising administering a composition comprising compositions according to claim 17.

19. The method of claim 18 wherein said immunization is prophylactic.

20. The method of claim 18 wherein said immunization is therapeutic.

L4 ANSWER 2 OF 26 USPATFULL on STN
2004:38115 Composition and methods of using hiv **vpr**.
Muthumani, Karrupiah, Upper Darby, PA, UNITED STATES
**Weiner, David B.**, Merion Station, PA, UNITED STATES
US 2004028651 A1 20040212
APPLICATION: US 2002-311260 A1 20021213 (10)
WO 2001-US10028 20010329
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of delivering a desired polypeptide to an individual are disclosed. The methods comprise administering to the individual an immunogenic vector comprising a nucleic acid encoding the desired polypeptide operably linked to regulatory elements in combination with one or more of **Vpr** protein, a functional fragment of **Vpr** protein, a nucleic acid encoding **Vpr** protein operably linked to regulatory elements, or a nucleic acid encoding fragment of **Vpr** protein operably linked to regulatory elements. Methods of inhibiting an undesirable immune response in an individual are disclosed. Methods for inhibiting the cellular proliferation of a tumor cell in an individual are disclosed.

CLM What is claimed is:
1. A method of delivering a desired polypeptide to an individual comprising administer to said individual: a) an immunogenic vector comprising a nucleic acid encoding the desired polypeptide operably linked to regulatory elements; and b) one or more of the components selected from the group consisting of: i) **Vpr** protein; ii) a functional fragment of **Vpr** protein; iii) a nucleic acid encoding **Vpr** protein operably linked to regulatory elements; and iv) a nucleic acid encoding a functional fragment of **Vpr** protein operably linked to regulatory elements.

2. The method of claim 1 wherein the individual is administered a nucleic acid encoding **Vpr** protein operably linked to regulatory elements.

3. The method of claim 2 wherein the nucleic acid encoding **Vpr** protein also encodes the desired polypeptide.

4. The method of claim 2 wherein a nucleic acid encoding **Vpr** protein and a nucleic acid encoding the desired polypeptide are administered to the individual in the same formulation.

5. The method of claim 4 wherein a nucleic acid encoding **Vpr** protein and a nucleic acid encoding the desired polypeptide are administered to the individual in separate formulations.

6. The method of claim 1 wherein the individual is administered **Vpr** protein.

7. The method of claim 6 wherein the **Vpr** protein and the nucleic acid encoding the desired polypeptide are administered in the same formulation.

8. The method of claim 6 wherein the **Vpr** protein and the nucleic acid encoding the desired polypeptide are administered in separate formulations.

9. The method of claim 1 wherein the desired polypeptide is a human polypeptide.

10. The method of claim 1 wherein the immunogenic vector is a viral vector.

11. The method of claim 10 wherein the viral vector is an adenoviral vector.

12. A composition comprising an immunogenic vector comprising a nucleic acid encoding the desired polypeptide operably linked to regulatory elements; and one or more of the components selected from the group consisting of: i) **Vpr** protein; ii) a functional fragment of **Vpr** protein; iii) a nucleic acid encoding **Vpr** protein operably linked to regulatory elements; and iv) a nucleic acid encoding a functional fragment of **Vpr** protein operably linked to regulatory elements.

13. The composition of claim 12 comprising a nucleic acid encoding **Vpr** protein operably linked to regulatory elements.

14. The composition of claim 13 comprising a nucleic acid that encodes **Vpr** protein and the desired polypeptide.

15. The composition of claim 13 comprising **Vpr** protein.

16. The composition of claim 15 wherein the **Vpr** protein is incorporated within the immunogenic vector.

17. The composition of claim 15 wherein the immunogenic vector is a viral vector.

18. The composition of claim 17 wherein the viral vector is an adenoviral vector.

19. A method for inhibiting an undesirable immune response in an individual comprising administering to said individual in an amount sufficient to inhibit an undesirable immune response one or more of the components selected from the group consisting of: i) **Vpr** protein; ii) a functional fragment of **Vpr** protein; iii) a nucleic acid encoding **Vpr** protein operably linked to regulatory elements; and iv) a nucleic acid encoding a functional fragment of **Vpr** protein operably linked to regulatory elements.

20. The method of claim 19 wherein the individual is administered a nucleic acid encoding **Vpr** protein operably linked to regulatory elements.

21. The method of claim 19 wherein the individual is administered **Vpr** protein.

22. The method of claim 19 wherein said individual has an autoimmune/inflammatory disease or condition.

22. The method of claim 19 wherein said individual is undergoing or has undergone a cell, tissue or organ transplant procedure.

23. The method of claim 19 wherein the undesirable immune response is septic shock.

24. The method of claim 23 wherein the component is a prior to the undesirable immune response and the treatment is prophylactic.

25. The method of claim 23 wherein the component is administered during the undesirable immune response and the treatment is therapeutic.

toxic shock.

27. The method of claim 26 wherein the component is administered prior to the desirable immune response and the treatment is prophylactic.

28. The method of claim 26 wherein the component is administered during the undesirable immune response and the treatment is therapeutic.

29. A method for inhibiting cellular proliferation in a tumor cell in an individual comprising administer to said individual, in an amount sufficient to inhibit cellular proliferation, a recombinant adenovirus comprising a nucleic acid encoding **Vpr** protein operably linked to regulatory elements or a nucleic acid encoding an anti-tumor fragment of **Vpr** protein operably linked to regulatory elements.

30. The method of claim 29 wherein the recombinant adenovirus comprises a nucleic acid encoding **Vpr** protein operably linked to regulatory elements.

31. The method of claim 29 herein the recombinant adenovirus comprises an anti-tumor fragment of **Vpr** protein operably linked to regulatory elements.

32. The method of claim 29 wherein the recombinant adenovirus is administered by intratumoral injection.

L4 ANSWER 3 OF 26 USPATFULL on STN
2003:332329 Compositions and methods for the abrogation of cellular proliferation utilizing the human immunodeficiency virus **VPR** protein.
**Weiner, David B.**, Merion, PA, United States
Levy, David N., Birmingham, AL, United States
Refaeli, Yosef, Boston, MA, United States
Williams, William V., Havertown, PA, United States
Ayyavoo, Velpandi, Havertown, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 6667157 B1 20031223
WO 9608970 19960328
APPLICATION: US 1997-809186 19970624 (8)
WO 1995-US12344 19950921
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Method of inhibiting proliferation of cells using **vpr** protein or nucleotide sequences that encode **vpr** are disclosed. Method of preventing lymphocyte activation using **vpr** protein or nucleotide sequences that encode **vpr** are disclosed. Methods of treating an individual diagnosed with or suspected of suffering from autoimmune disease, diseases characterized by proliferating cells and graft versus host disease by administering **vpr** protein or a functional fragment thereof, or a nucleic acid molecule that comprises a nucleotide sequence that encodes **vpr** protein or a functional fragment thereof are disclosed. Conjugated compositions for delivery of active agents to the nucleus of cells are disclosed.
CLM What is claimed is:
1. A method of inhibiting proliferation of cells which comprises the steps a) obtaining isolated **Vpr** protein or a function fragment thereof; and b) contacting cells with an amount of said **Vpr** protein or functional fragment thereof effective to inhibit cell proliferation, wherein said cells are T cells and/or B cells and/or monocytes.

2. The method of claim 1 which comprises the step of: contacting cells with **Vpr** protein.

3. The method of claim 2 wherein said T cells and/or B cells and/or

monocytes are removed from an individual prior to being contacted with **Vpr** protein.

4. The method of claim 1 which comprises the step of: contacting cells with a functional fragment of **Vpr** protein.

5. The method of claim 4 wherein said T cells and/or B cells-and/or monocytes are removed from an individual prior to being contacted with said functional fragment of **Vpr** protein.

6. The method of claim 3 wherein said cells are T cells.

7. The method of claim 4 wherein said cells are monocytes.

8. The method of claim 3 wherein said cells are monocytes.

9. The method of claim 4 wherein said cells are T cells.

L4 ANSWER 4 OF 26 USPATFULL on STN
2003:294238 **VPR** function and activity.
**Weiner, David B.**, Merion, PA, UNITED STATES
Levy, David Nathan, Philadelphia, PA, UNITED STATES
Refaeli, Yosef, Philadelphia, PA, UNITED STATES
US 2003207252 A1 20031106
APPLICATION: US 2001-935100 A1 20010822 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Pharmaceutical compositions comprising the HIV protein **vpr** or nucleic acid molecule encoding **vpr** are disclosed. Also disclosed are methods of treating patients suffering from diseases characterized by hyperproliferating undifferentiated cells such as cancer by administering such compositions. Methods of identifying compounds which have anti-HIV activity are disclosed, in particular, methods of identifying compounds which modulate the activity of **vpr** and of identifying compounds which inhibit **vpr** binding to the HIV protein gag.

CLM What is claimed is:

1. A method of inducing undifferentiated cells to differentiate which comprises the step of: contacting undifferentiated cells with an amount of **vpr** protein or a functional fragment thereof effective to stimulate differentiation; or introducing into undifferentiated cells a nucleic acid molecule that comprises a nucleotide sequence that encodes **vpr** protein or a functional fragment thereof whereby said nucleotide sequence is expressed by said cells.

2. A pharmaceutical composition comprising a) **vpr** protein or a functional fragment thereof, or a nucleic acid molecule that comprises a nucleotide sequence that encodes **vpr** protein or a functional fragment thereof; and b) pharmaceutically acceptable carrier.

3. A method of treating an individual diagnosed with or suspected of suffering from diseases characterized by hyperproliferating undifferentiated cells which comprises the step of administering to said individual an effective amount of a pharmaceutical composition according to claim 2.

4. A pharmaceutical composition that comprises redifferentiated tumor cells induced to redifferentiate by contacting tumor cells with **vpr** protein or introducing into tumor cells a nucleic acid molecule that comprises a nucleotide sequence that encodes **vpr** protein.

5. A method of treating an individual suffering from a disease associated with the loss or disfunction of cells which comprises the step of implanting into said individual a pharmaceutical composition according to claim 4.

6. A method of identifying compounds which inhibit **vpr** from stimulating differentiation of undifferentiated cells which comprises the steps of a) contacting, in the presence of a test compound, said undifferentiated cells with an amount of **vpr** protein sufficient to stimulate differentiation and b) comparing the differentiation that occurs with the differentiation that occurs when said undifferentiated cells are contacted with **vpr** protein in the absence of said test compound.

7. A kit for performing the method of identifying compounds which inhibit **vpr** from stimulating differentiation of undifferentiated cells of claim 6, said kit comprising a) a first container comprising undifferentiated cells, and b) a second container comprising **vpr** protein.

8. A method of identifying compounds that inhibit **vpr** protein binding to p55, p24, p15, p7 or p6 protein which comprises the steps of: a) contacting **vpr** protein or a fragment thereof and p55, p24, p15, p7 or p6 protein or a fragment thereof in the presence of a test compound, b) determining the level of binding between **vpr** protein and p55, p24, p15, p7 or p6 protein and c) comparing that level to the level of binding that occurs when **vpr** protein or a fragment thereof and p55, p24, p15, p7 or p6 protein or a fragment thereof are contacted in the absence of a test compound.

9. A kit for performing the method of identifying compounds which inhibit **vpr** protein binding to p55, p24, p15, p7 or p6 protein of claim 8, said kit comprising: a) a first container comprising **vpr** protein or a fragment thereof and b) a second container comprising p55, p24, p15, p7 or p6 protein or a fragment thereof.

10. A method of identifying compounds that inhibit p24 protein binding to p15 or p7 protein which comprises the steps of: a) contacting p24 protein or a fragment thereof and p15 or p7 protein or a fragment thereof in the presence of a test compound, b) determining the level of binding between p24 protein and p15 or p7 protein and c) comparing that level to the level of binding that occurs when p24 protein or a fragment thereof and p15 or p7 protein or a fragment thereof are contacted in the absence of a test compound.

11. A kit for performing the method of identifying compounds which inhibit p24 protein binding to p15 or p7 protein of claim 10, said kit comprising: a) a first container comprising p24 protein or a fragment thereof and b) a second container comprising p15 or p7 protein or a fragment thereof.

12. A method of identifying compounds which inhibit p24 aggregation which comprises the steps of: a) maintaining p24 protein under conditions which promote its aggregation in the presence of a test compound, b) determining the level of p24 aggregation and c) comparing that level to the level of aggregation that occurs when p24 protein is maintained under the same conditions in the absence of a test compound.

13. A kit for performing the method of identifying compounds which inhibit p24 aggregation of claim 12, said kit comprising a) a first container comprising p24 protein and b) a second container comprising p15 protein or MAb 1238.

14. Isolated **antibodies** which specifically bind to **vpr** protein produced in eukaryotic cells.

15. A method of identifying an individual exposed to HIV comprising the steps of: a) contacting a sample with **antibodies** according to claim 14, and b) detecting whether said **antibodies** are bound to **vpr**.

16. A kit for identifying individuals exposed to HIV comprising a) a

first container comprising **antibodies** according to claim 17, and b) a second container which contains **vpr** protein produced in eukaryotic cells.

17. Isolated **vpr** protein produced in eukaryotic cells.

18. A method of identifying an individual exposed to HIV comprising the steps of: a) contacting a sample with **vpr** protein according to claim 17, and b) detecting whether said **vpr** is bound to **antibodies**.

19. A kit for identifying individuals exposed to HIV comprising a) a first container comprising **vpr** protein according to claim 17, and b) a second container which contains **antibodies** which specifically bind to **vpr** protein produced in eukaryotic cells.

20. A method of enhancing retroviral propagation in cell culture comprising the step of: adding **vpr** protein in conjunction with infection of the cells by retrovirus; or introducing into a nucleic acid molecule that comprises a sequence that encodes **vpr** protein in conjunction with infecting said cells with a retrovirus.

21. A method of identifying compounds that inhibit **vpr** enhancement of retroviral replication comprising the steps of: a) infecting cells with a retrovirus in the presence of **vpr** protein and a test compound or infecting with a retrovirus in the presence of **vpr** protein, cells transformed with a nucleic acid molecule that comprises a nucleotide sequence that encodes **vpr**, wherein the transformed cells produce **vpr** protein and b) comparing the amount of virus produced with the amount of virus produced by infecting cells with a retrovirus in the absence of a test compound.

22. A method of modifying macrophage cells comprising the step of contacting macrophage cells with **vpr** protein or introducing into the macrophage cells a nucleic acid molecule that comprises a sequence that encodes **vpr** protein.

23. A method of treating individuals diagnosed with or suspected of suffering from diseases characterized by undesirable activity of macrophage cells comprising the step of administering to such individuals, an effective amount of the pharmaceutical composition of claim 2.

24. Drug delivery particles comprising **vpr**, p24 and a non-HIV, cell-type specific envelope protein.

25. A method of delivering **vpr** to cells comprising administering a drug delivery particle of claim 24.

26. A fusion compound comprising a biologically active portion linked to a **vpr** fragment which binds to p24.

27. Drug delivery particles comprising a fusion compound of claim 26, p24 and a cell-type specific envelope protein.

28. A nucleic acid molecule that comprises a nucleotide sequence that encodes a fusion compound of claim 26.

29. An expression vector that comprises a nucleic acid molecule of claim 28.

30. A host cell that comprises an expression vector of claim 29.

31. A method of delivering a fusion compound to cells comprising administering a drug delivery particle of claim 27 wherein said fusion compounds comprised a biologically active portion linked to a **vpr** fragment which binds to p24

32. A pharmaceutical composition comprising a) **vpr**, an immunogenic fragment of **vpr** or anti-**vpr antibodies**; and b) a pharmaceutically acceptable carrier.

33. A method of treating an individual exposed to HIV by administering an immunogenic amount of **vpr**, an immunogenic fragment of **vpr** or an effective amount of anti-**vpr antibodies**.

L4 ANSWER 5 OF 26 USPATFULL on STN
2003:207377 Cellular receptor for HIV-1 **Vpr** essential for G2/M phase transition of the cell cycle.
**Weiner, David B.**, Merion Station, PA, UNITED STATES
Ayyavoo, Velpandi, Monroeville, PA, UNITED STATES
Mahalingam, Sundarasamy, Birmingham, AL, UNITED STATES
Patel, Mamata, Philadelphia, PA, UNITED STATES
US 2003143735 A1 20030731
APPLICATION: US 2003-208338 A1 20030227 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods of identifying an anti-HIV compound by contacting human **Vpr** Interacting Protein (hVIP), or a fragment thereof known to interact with **Vpr**, with **Vpr**, or a fragment thereof known to interact with hVIP in the presence of a test compound, and comparing the affinity of the hVIP or fragment thereof to the **Vpr** or fragment thereof in the presence of the test compound with the affinity of the hVIP or fragment thereof to the **Vpr** or fragment thereof in the absence of the test compound. The present invention also provides transgenic non-human mammals comprising a recombinant expression vector that comprises a nucleic acid sequence that encodes hVIP.
CLM What is claimed is:
1. A substantially pure protein having the amino acid sequence of SEQ ID NO:2.

2. A recombinant expression vector comprising a nucleic acid sequence that encodes a protein of claim 1.

3. The recombinant expression vector of claim 2 comprising SEQ ID NO:1.

4. A host cell comprising the recombinant expression vector of claim 2.

5. The host cell of claim 4 comprising a recombinant expression vector that comprises SEQ ID NO:1.

6. An isolated nucleic acid molecule consisting of SEQ ID NO:1, or a fragment thereof having at least 10 nucleotides.

7. The nucleic acid molecule of claim 6 consisting of SEQ ID NO:1.

8. The nucleic acid molecule of claim 6 consisting of a fragment of SEQ ID NO:1 having at least 10 nucleotides.

9. The nucleic acid molecule of claim 6 consisting of a fragment of SEQ ID NO:1 having 12-150 nucleotides.

10. The nucleic acid molecule of claim 6 consisting of a fragment of SEQ ID NO:1 having 15-50 nucleotides.

11. The nucleic acid molecule of claim 6 consisting of a fragment of SEQ ID NO:1 having 18-30 nucleotides.

12. The nucleic acid molecule of claim 6 consisting of a fragment of SEQ ID NO:1 having 24 nucleotides.

13. An oligonucleotide molecule comprising a nucleotide sequence complementary to a nucleotide sequence of at least 10 nucleotides of SEQ ID NO:1.

14. The oligonucleotide molecule of claim 13 consisting of a nucleotide sequence complementary to a nucleotide sequence of 10-150 nucleotides of SEQ ID NO:1.

15. The oligonucleotide molecule of claim 13 consisting of a nucleotide sequence complementary to a nucleotide sequence of 18-28 nucleotides of SEQ ID NO:1.

16. An isolated **antibody** which binds to an epitope on a protein of claim 1.

17. The **antibody** of claim 16 wherein said **antibody** is a monoclonal **antibody**.

18. A pharmaceutical composition comprising a nucleic acid molecule of claim 6 and a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising an oligonucleotide of claim 13 and a pharmaceutically acceptable carrier.

20. A method of making human **Vpr** Interacting Protein comprising: isolating nucleic acid molecule having SEQ ID NO:1; inserting said nucleic acid molecule into an expression vector; inserting said expression vector into host cell under conditions in which said protein is expressed; and isolating said human **Vpr** Interacting Protein.

21. A method of inhibiting the expression of human **Vpr** Interacting Protein In a cell comprising contacting said cell with an oligonucleotide complementary to SEQ ID NO:1, or a fragment thereof, whereby said oligonucleotide inhibits expression of said protein.

22. The method of claim 21 wherein said cell is a cancer cell in an animal.

23. The method of claim 21 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 10-150 nucleotides of SEQ ID NO:1.

24. The method of claim 21 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 18-28 nucleotides of SEQ ID NO:1.

25. A method of treating an individual who has cancer comprising administering to said individual a therapeutically effective amount of an oligonucleotide complementary to SEQ ID NO:1, or a fragment thereof, whereby said oligonucleotide inhibits expression of said protein.

26. The method of claim 25 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 10-150 nucleotides of SEQ ID NO:1.

27. The method of claim 25 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 18-28 nucleotides of SEQ ID NO:1.

28. A method of inhibiting human **Vpr** Interacting Protein activity in a cell comprising contacting said cell an hVIP-binding fragment thereof.

29. The method of claim 28 wherein said cell is in an individual who has cancer and said method comprises the step of administering to said individual a therapeutically effective amount of an hVIP-binding fragment of **Vpr**.

L4 ANSWER 6 OF 26 USPATFULL on STN
2002:294310 Compositions and methods of using capsid protein from Flaviviruses and Pestiviruses.
**Weiner, David B.**, Merion Station, PA, UNITED STATES
Yang, Joo-Sung, Philadelphia, PA, UNITED STATES
US 2002164349 A1 20021107
APPLICATION: US 2001-971980 A1 20011004 (9)
PRIORITY: US 2000-237885P 20001004 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods of inducing cell death with Flavivirus or Pestivirus capsid protein, such as West Nile virus (WNV) capsid protein, and functional fragments thereof. The invention also provides methods of treating patients suffering from diseases characterized by hyperproliferating cells by administering pharmaceutical compositions comprising WNV or other virus including Flavivirus or Pestivirus capsid or other protein or a nucleic acid molecule encoding the same. Methods of identifying compounds which have anti-viral and/or anti-WNV and/or anti-Flavivirus and/or anti-Pestivirus capsid or other protein activity are disclosed. The invention also provides vaccine compositions comprising capsid or other proteins, or fragments thereof, or nucleic acids encoding same, from WNV or other virus including Flavivirus or Pestivirus and a pharmaceutically acceptable carrier. The invention also provides diagnostic methods and kits for identifying individuals exposed to WNV or other viruses including Flavivirus or Pestivirus.

CLM What is claimed is:

1. A method of inducing cell death comprising the step of contacting a cell with an amount of isolated Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, effective to induce cell death; or introducing into said cell a nucleic acid molecule comprising a nucleotide sequence encoding a Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, said nucleic acid being free from an entire Flavivirus or Pestivirus virus genome, wherein said nucleotide sequence is expressed in said cell at a level effective to induce cell death.

2. The method of claim 1, wherein the isolated capsid protein, or functional fragment thereof, or the nucleic acid molecule is from a virus selected from the Japanese encephalitis virus group subgenus.

3. The method of claim 1, wherein the isolated capsid protein, or functional fragment thereof, or the nucleic acid molecule is from West Nile virus (WNV).

4. The method of claim 3, wherein the functional fragment comprises SEQ ID NO:8.

5. The method of claim 3, wherein the nucleic acid molecule encodes SEQ ID NO:8.

6. The method of claim 1, wherein the cell is a tumor cell.

7. The method of claim 1, wherein the cell is contacted with the Flavivirus or Pestivirus capsid protein, or a functional fragment thereof.

8. The method of claim 1, wherein the nucleic acid molecule is introduced into said cell.

9. A method of identifying compounds that inhibit Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, from inducing apoptosis in cells comprising the steps of a) contacting the cells, in the presence of a test compound, with an amount of Flavivirus or Pestivirus capsid protein, or a functional fragment thereof,

sufficient to induce a detectable level of apoptosis in the cells; and b) comparing the level of apoptosis detected in step (a) with the level of apoptosis that occurs when cells are contacted with Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, in the absence of said test compound.

10. The method of claim 9, wherein the cells are contacted with Flavivirus or Pestivirus capsid protein.

11. The method of claim 9, wherein the cells are contacted with a functional fragment of Flavivirus or Pestivirus capsid protein.

12. The method of claim 11, wherein the functional fragment comprises SEQ ID NO:8.

13. The method of claim 9, wherein the cells are selected from the group consisting of Hela cells, RD cells, and 293 cells.

14. The method of claim 9, wherein the detecting step is an assay that detects a marker of apoptosis.

15. The method of claim 14, wherein the marker is phosphatidylserine (PS) or free 3'-hydroxy DNA termini.

16. The method of claim 15, wherein the assay is TUNEL analysis or annexin V flow cytometry.

17. A kit for performing the method of claim 9 comprising a) a container comprising Flavivirus or Pestivirus capsid protein, or functional fragment thereof; and b) at least one additional component selected from the group consisting of: instructions, positive controls, negative controls, photos depicting data, and figures depicting data.

18. An injectable pharmaceutical composition comprising a) a Flavivirus or Pestivirus capsid protein, or a functional fragment thereof, or a nucleic acid molecule that comprises a nucleotide sequence that encodes a Flavivirus or Pestivirus capsid protein or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

19. The injectable pharmaceutical composition of claim 18 comprising a) a nucleic acid molecule that comprises a nucleotide sequence that encodes a Flavivirus or Pestivirus capsid protein or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

20. The injectable pharmaceutical composition of claim 18 comprising a) a Flavivirus or Pestivirus capsid protein, or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

21. The injectable pharmaceutical composition of claim 18 comprising a) a WNV capsid protein, or a functional fragment thereof; and b) a pharmaceutically acceptable carrier.

22. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by hyperproliferating cells which comprises the step of administering to said individual an effective amount of the injectable pharmaceutical composition of claim 18.

23. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by hyperproliferating cells which comprises the step of administering to said individual an effective amount of the injectable pharmaceutical composition of claim 19.

24. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by hyperproliferating cells which comprises the step of administering to said individual an effective amount of the injectable pharmaceutical composition of claim 20.

25. A method of treating an individual diagnosed with or suspected of suffering from a disease characterized by undesirable cells comprising eliminating the undesirable cells by administering to said individual an effective amount of the injectable pharmaceutical composition of claim 18.

26. The method of claim 24, wherein the capsid protein, or functional fragment thereof, is WNV capsid protein, or functional fragment thereof.

27. The method of claim 22, wherein the disease is cancer.

28. The method of claim 22, wherein the administration step is accomplished by intra-tumoral injection of the injectable pharmaceutical composition.

29. A method of identifying an individual exposed to Flavivirus or Pestivirus comprising the steps of: a) contacting **antibodies** specific for Flavivirus or Pestivirus capsid protein with a sample from the individual; and b) detecting whether said **antibodies** are bound to Flavivirus or Pestivirus capsid protein from the sample, wherein detection of binding of the **antibodies** to Flavivirus or Pestivirus capsid protein is indicative of exposure of the individual to Flavivirus or Pestivirus.

30. The method of claim 24, wherein the capsid protein is WNV capsid protein.

31. A kit for identifying individuals exposed to a Flavivirus or Pestivirus comprising a) a first container comprising **antibodies** specific for a Flavivirus or Pestivirus capsid protein; and b) a second container comprising Flavivirus or Pestivirus capsid protein, or a fragment thereof.

32. The kit of claim 31, wherein the first container comprises **antibodies** specific for WNV capsid protein and the second container comprises WNV capsid protein, or a fragment thereof.

33. A method of identifying an individual exposed to a Flavivirus or Pestivirus comprising the steps of: a) contacting a sample with Flavivirus or Pestivirus capsid protein; and b) detecting whether said Flavivirus or Pestivirus capsid protein is bound to **antibodies** in said sample, wherein detection of binding of Flavivirus or Pestivirus capsid protein is indicative of exposure of the individual to Flavivirus or Pestivirus.

34. The method of claim 33, wherein the virus is WNV and the capsid protein is WNV capsid protein.

35. A kit for identifying individuals exposed to a Flavivirus or Pestivirus comprising a) a first container comprising Flavivirus or Pestivirus capsid protein; and b) a second container which contains **antibodies** which specifically bind to Flavivirus or Pestivirus capsid protein.

36. The kit of claim 35, wherein the capsid protein is WNV capsid protein.

37. A vaccine composition comprising a) an immunologically effective amount of Flavivirus or Pestivirus capsid protein, or an immunogenic fragment thereof; and b) a pharmaceutically acceptable carrier.

38. The vaccine of claim 37, wherein the Flavivirus or Pestivirus capsid protein, or immunogenic fragment thereof, is WNV capsid protein, or immunogenic fragment thereof.

39. A vaccine composition comprising a) nucleic acid encoding Flavivirus or Pestivirus capsid protein, or an immunogenic fragment thereof, and b) a pharmaceutically acceptable carrier.

40. The vaccine of claim 39, wherein the nucleic acid encodes WNV capsid protein, or an immunogenic fragment thereof.

41. A method of treating an individual exposed to a Flavivirus or Pestivirus by administering a therapeutically effective amount of capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus, or a nucleic acid encoding capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus.

42. The method of claim 41, wherein the virus to which the individual is exposed is WNV, and wherein the capsid protein, or fragment thereof, or the nucleic acid encoding the capsid protein, or immunogenic fragment thereof, is from WNV.

43. A method of protecting an individual from Flavivirus or Pestivirus infection by administering a prophylactically effective amount of capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus, or a nucleic acid encoding capsid protein, or an immunogenic fragment thereof, from a Flavivirus or Pestivirus.

44. The method of claim 43, wherein the virus against which the individual is to be protected is WNV, and wherein the capsid protein, or fragment thereof, or the nucleic acid encoding the capsid protein, or immunogenic fragment thereof, is from WNV.

L4 ANSWER 7 OF 26 USPATFULL on STN
2002:276069 Genetic immunization.
**Weiner, David B.**, Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 6468982 B1 20021022
APPLICATION: US 1997-880576 19970623 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being, expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:
1. A method for inducing an immune response in an individual against an antigen comprising orally administering to said individual, a pharmaceutical composition comprising bupivacaine and DNA molecules that comprise a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are free from viral particles and when said DNA molecules are taken up by cells of said individual, said DNA sequence is expressed in said cells and an immune

response is generated against said antigen.

2. The method of claim 1 wherein said DNA molecules are plasmids.

3. The method of claim 1 wherein said pharmaceutical composition consists essentially of DNA molecules and bupivacaine.

4. The method of claim 1 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

5. The method of claim 4 wherein said pathogen is an intracellular pathogen.

6. A method of immunizing an individual against a Herpes simplex 2 virus (HSV2) antigen comprising orally administering to said individual, a pharmaceutical composition comprising bupivacaine and DNA molecules that comprise a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are free of viral particles and when said DNA molecules are taken up by cells of said individual, said DNA sequence is expressed in said cells and a protective immune response is generated against said antigen.

7. A method of inducing an immune response in an individual against a pathogen comprising orally administering to said individual, a pharmaceutical composition comprising bupivacaine and DNA molecules that comprise a DNA sequence that encodes an antigen from said pathogen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are free of viral particles and when said DNA molecules are taken up by cells of said individual, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

8. The method of claim 7 wherein said DNA molecules are plasmids.

9. The method of claim 7 wherein said pharmaceutical composition consists essentially of DNA molecules and bupivacaine.

10. The method of claim 7 wherein said pathogen is an intracellular pathogen.

11. The method of claim 7 wherein said pathogen is a virus.

12. A method of immunizing an individual against a Herpes simplex 2 virus (HSV2) comprising orally administering to said individual, a pharmaceutical composition comprising bupivacaine and DNA molecules that comprise a DNA sequence that encodes an antigen from said pathogen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are free of viral particles and when said DNA molecules are taken up by cells of said individual, said DNA sequence is expressed in said cells and a protective immune response is generated against said antigen.

13. A method of treating an individual who is infected by Herpes simplex 2 virus (HSV2) comprising orally administering to said individual, a pharmaceutical composition comprising bupivacaine and DNA molecules that comprise a DNA sequence that encodes an antigen from HSV2, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are free from viral particles and when said DNA molecules are taken up by cells of said individual, said DNA sequence is expressed in said cells and a therapeutically effective immune response is generated.

14. The method of claim 13 wherein said DNA molecule is a plasmid.

15. The method of claim 13 wherein said pharmaceutical composition

consists essentially of DNA molecules and equivalents.

L4 ANSWER 8 OF 26 USPATFULL on STN
2002:259414 Methods of inducing mucosal immunity.
**Weiner, David B.**, Merion, PA, UNITED STATES
Wang, Bin, Havertown, PA, UNITED STATES
Ugen, Kenneth E., Philadelphia, PA, UNITED STATES
The trustees of the University of Pennsylvania (U.S. corporation)
US 2002142987 A1 20021003
APPLICATION: US 2002-76900 A1 20020214 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of inducing mucosal immunity in individuals against proteins and peptides are disclosed. The methods comprise the step of administering topically or by lavage into mucosal tissue selected from the group consisting of rectal, vaginal, urethral, sublingual and buccal, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein or peptide that comprises an epitope against which mucosal immunity is desired. The methods may be used to immunize and individual against a pathogen infection, hyperproliferative diseases or autoimmune diseases using nucleic acid molecules which encode proteins and peptides that share an epitope with a pathogen antigen or protein associated with cells involved in hyperproliferative diseases or autoimmune diseases, respectively,

CLM What is claimed is:

1. A method of inducing mucosal immunity against proteins and peptides in an individual comprising the step of administering by topical or lavage administration to mucosal tissue of said individual, a nucleic acid molecule that comprises a nucleotide sequence that either encodes a desired peptide or protein, wherein said mucosal tissue is selected from the group consisting of rectal, vaginal, urethral, sublingual and buccal.

2. The method of claim 1 wherein said nucleic acid molecule comprises a nucleotide sequence that encodes a protein and is operably linked to regulatory sequences.

3. The method of claim 1 wherein said nucleic acid molecule comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of an antigen against which an immune response is desired, said nucleotide sequence operably linked to regulatory sequences.

4. The method of claim 1 wherein said nucleic acid molecule is administered rectally.

5. The method of claim 1 wherein said nucleic acid molecule is administered vaginally.

6. The method of claim 1 wherein said nucleic acid molecule is administered sublingually.

7. The method of claim 1 wherein said nucleic acid molecule is administered into buccal tissue.

8. A method of immunizing an individual against a pathogen comprise the step of inducing mucosal immunity against a pathogen antigen in an individual by administering by topical or lavage administration to the mucosal tissue of said individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a peptide which comprises at least an epitope identical or substantially similar to an epitope displayed on a pathogen antigen, wherein said mucosal tissue is selected from the group consisting of rectal, vaginal, urethral, sublingual and buccal.

9. The method of claim 8 wherein said nucleic acid molecule comprises a nucleotide sequence that encodes a protein and is operably linked to regulatory sequences.

10. The method of claim 8 wherein said nucleic acid molecule comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of an antigen against which an immune response is desired, said nucleotide sequence operably linked to regulatory sequences.

11. The method of claim 8 wherein said nucleic acid molecule is administered rectally.

12. The method of claim 8 wherein said nucleic acid molecule is administered vaginally.

13. The method of claim 8 wherein said nucleic acid molecule is administered sublingually.

14. The method of claim 8 wherein said nucleic acid molecule is administered into buccal tissue.

L4 ANSWER 9 OF 26 USPATFULL on STN
2002:230844 Cellular receptor for HIV-1 **Vpr** essential for G2/M phase transition of the cell cycle.
**Weiner, David B.**, Merion Station, PA, United States
Ayyavoo, Velpandi, Monroeville, PA, United States
Mahalingam, Sundarasamy, Birmingham, AL, United States
Patel, Mamata, Philadelphia, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 6448078 B1 20020910
WO 9919359 19990422
APPLICATION: US 2000-529245 20001017 (9)
WO 1998-US21432 19981009 20001017 PCT 371 date
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to substantially pure Human **Vpr** Interacting Protein (hVIP), and fragments thereof. Also disclosed are isolated nucleic acid molecules that encode hVIP, or a fragment thereof; nucleic acid probes and primers directed to nucleic acid molecules that encode hVIP, or a fragment thereof; oligonucleotide molecules that consist of a nucleotide sequence complementary to a portion of the nucleotide sequence that encodes hVIP; vectors comprising nucleic acid molecules encoding hVIP; recombinant expression vectors that comprise nucleic acid sequences that encode hVIP; host cells that comprise recombinant expression vectors which include nucleic acid sequences that encode hVIP; genetic therapy vectors comprising nucleic acid molecules encoding hVIP; isolated **antibody** which binds to an epitope on hVIP; pharmaceutical compositions comprising a pharmaceutically acceptable carrier and nucleic acid molecules complementary to a portion of hVIP; methods of making hVIP; and methods of inhibiting expression of hVIP oligonucleotides complementary to a portion of the nucleotide sequence that encodes hVIP.
CLM What is claimed is:
1. An isolated **antibody** which binds to an epitope on a protein having the amino acid sequence of SEQ ID NO:2.

2. The **antibody** of claim 1 wherein said **antibody** is a monoclonal **antibody**.

3. A method of inhibiting the expression of human **Vpr** Interacting Protein in a cell comprising contacting said cell with an oligonucleotide complementary to SEQ ID NO:1, or a fragment thereof, whereby said oligonucleotide inhibits expression of said protein.

4. The method of claim 3 wherein said cell is a cancer cell in an animal.

5. The method of claim 3 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 10-150 nucleotides of SEQ ID NO:1.

6. The method of claim 3 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 18-28 nucleotides of SEQ ID NO:1.

7. A method of treating an individual who has cancer comprising administering to said individual a therapeutically effective amount of an oligonucleotide complementary to SEQ ID NO:1, or a fragment thereof, whereby said oligonucleotide inhibits expression of said protein.

8. The method of claim 7 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 10-150 nucleotides of SEQ ID NO:1.

9. The method of claim 7 wherein said oligonucleotide consists of a nucleotide sequence complementary to a nucleotide sequence of 18-28 nucleotides of SEQ ID NO:1.

10. A method of inhibiting human **Vpr** Interacting Protein activity in a cell comprising contacting said cell an hVIP-binding fragment thereof.

11. The method of claim 10 wherein said cell is in an individual who has cancer and said method comprises the step of administering to said individual a therapeutically effective amount of an hVIP-binding fragment of **Vpr**.

L4 ANSWER 10 OF 26 USPATFULL on STN
2002:34422 Methods of inducing mucosal immunity.
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Wang, Bin, Havertown, PA, United States
Ugen, Kenneth E., Philadelphia, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 6348449 B1 20020219
APPLICATION: US 1994-357398 19941216 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of inducing mucosal immunity in individuals against proteins and peptides are disclosed. The methods comprise the step of administering topically or by lavage into mucosal tissue selected from the group consisting of rectal, vaginal, urethral, sublingual and buccal, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein or peptide that comprises an epitope against which mucosal immunity is desired. The methods may be used to immunize an individual against a pathogen infection, hyperproliferative diseases or autoimmune diseases using nucleic acid molecules which encode proteins and peptides that share an epitope with a pathogen antigen or protein associated with cells involved in hyperproliferative diseases or autoimmune diseases, respectively.
CLM What is claimed is:
1. A method of inducing a mucosal immune response against an antigen in an individual comprising the step of administering by topical or lavage administration to mucosal tissue of said individual, a composition comprising bupivacaine and a DNA molecule that comprises a nucleotide sequence that encodes said antigen, said nucleotide sequence operatively linked to regulatory sequences which control the expression of said DNA sequence, wherein: said mucosal tissue is selected from the group consisting of rectal, vaginal, urethral, sublingual and buccal; said DNA

molecule is taken up by cells, said DNA sequence is expressed in said cells and a mucosal immune response is generated against said antigen.

2. The method of claim 1 wherein said DNA molecule is administered rectally.

3. The method of claim 1 wherein said DNA molecule is administered sublingually.

4. The method of claim 1 wherein said DNA molecule is administered into buccal tissue.

5. The method of claim 1 wherein said composition further comprises a DNA molecule which comprises a nucleotide sequence that encodes: a cytokine operatively linked to regulatory sequences which control the expression of said DNA sequence; and/or a nucleotide sequence that encodes a lymphokine, said nucleotide sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

6. The method of claim 1 wherein said composition comprises a DNA molecule which comprises a nucleotide sequence that encodes a protein operatively linked to regulatory sequences which control the expression of said DNA sequence, wherein said protein is selected form the group consisting of α-interferon, gamnma-interferon, platelet derived growth factor (PDGF), GC-SF, GM-CSF, TNF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12.

7. A method of inducing a mucosal immune response against an antigen in an individual comprising the step of administering to said individual by intravaginal topical or lavage administration, a composition comprising bupivacaine and a DNA molecule that comprises a nucleotide sequence that encodes said antigen, said nucleotide sequence operatively linked to regulatory sequences which control the expression of said DNA sequence, wherein: said DNA molecule is administered free of an infectious agent; and said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and a mucosal immune response is generated against said antigen.

8. The method of claim 7 wherein said composition further comprises a DNA molecule which comprises a nucleotide sequence that encodes: a cytokine operatively linked to regulatory sequences which control the expression of said DNA sequence; and/or a nucleotide sequence that encodes a lymphokine, said nucleotide sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

9. The method of claim 8 wherein said composition further comprises a DNA molecule which comprises a nucleotide sequence that encodes a protein operatively linked to regulatory sequences which control the expression of said DNA sequence, wherein said protein is selected form the group consisting of α-interferon, gamma-interferon, platelet derived growth factor (PDGF), GC-SF, GM-CSF, TNF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12.

L4 ANSWER 11 OF 26 USPATFULL on STN
2001:33252 Compositions and methods for delivery of genetic material.
Carrano, Richard A., Paoli, PA, United States
Wang, Bin, Haidian, China
**Weiner, David B.**, Merion, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)Apollan, Inc., Malvern, PA, United States (U.S. corporation)
US 6197755 B1 20010306
APPLICATION: US 1999-321461 19990527 (9)
DOCUMENT TYPE: Utility; Granted.

AB Methods of introducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a genetic vaccine facilitator and administering to the cells, a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produce a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:

1. A method of introducing nucleic acid molecules into cells of an individual comprising the step of administering in vivo to cells in said individual's body urea and nucleic acid molecules wherein said nucleic acid molecules are taken up by said cells.

2. The method of claim 1 wherein said nucleic acid molecules are administered to cells in said individual's body cells by intramuscular administration.

3. The method of claim 1 wherein said nucleic acid molecules are DNA molecules.

4. The method of claim 3 wherein said DNA molecules are plasmids.

5. A method of generating an immune response in an individual against an antigen comprising administering in vivo to cells of said individual's body urea and DNA molecules that comprise a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

6. The method of claim 5 wherein said DNA molecules are plasmids.

7. The method of claim 5 wherein said DNA molecules are administered to cells in said individual's body cells by intramuscular administration.

8. The method of claim 5 wherein said DNA molecules are administered to cells in said individual's body cells by administration into skin.

9. The method of claim 5 wherein said urea and said DNA molecules are administered simultaneously.

10. The method of claim 5 wherein said antigen is a pathogen antigen.

11. The method of claim 5 wherein said antigen is a protein associated with cells that characterize a disease.

12. The method of claim 10 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus, HAV; hepatitis B virus, HBV; hepatitis C virus, HCV; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBV; rhinovirus; and, coronavirus.

13. The method of claim 11 wherein said disease is characterized by hyperproliferating cells.

14. The method of claim 11 wherein said disease is an autoimmune

disease.

15. The method of claim 13 wherein said protein is selected from the group consisting of: protein products of oncogenes, myb, myc, fyn, ras, sarc, neu and trk; protein products of translocation gene bcl/abl; p53; EGRF; variable regions of **antibodies** made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

16. The method of claim 14 wherein said protein is selected from the group consisting of: variable regions of **antibodies** involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmunc disease.

17. A pharmaceutical composition comprising urea and DNA molecules that comprise a nuclcotide sequence which encodes a protein wherein said nucleotide sequence is operably linked to regulatory sequences required for expression in a mammal.

18. The pharmaceutical composition of claim 17 wherein said protein is selected from the group consisting of: pathogen antigens, proteins associated with hyperproliferating cells; and proteins associated with cells that characterize an autoimmunc disease.

19. A pharmaceutical kit comprising: i) a container that comprises DNA molecules that comprise a nucleotide sequence which encodes a protein wherein said nucleotide sequence is operably linked to regulatory sequences required for expression in a mammal; and ii) a container that comprises urea.

20. The pharmaceutical kit of claim 19 wherein said protein is selected from the group consisting of: pathogen antigens, proteins associated with hyperproliferating cells; and proteins associated with cells that characterize an autoimmunc disease.

21. A method of delivering a protein into cells of an individual in vivo comprising administering to cells of said individual's body, urea and DNA molecules that comprise a DNA sequence that encodes said protein, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecules are taken up by cells, said DNA sequence is expressed in said cells producing said protein in said cells.

22. The method of claim 21 wherein said DNA molecules are plasmids.

23. The method of claim 21 wherein said DNA molecules are administered to cells in said individual's body cells by intramuscular administration.

24. The method of claim 21 wherein said DNA molecules are administered to cells in said individual's body cells by administration into skin.

L4 ANSWER 12 OF 26 USPATFULL on STN
2001:4875 Cellular receptor for HIV-1 **Vpr** essential for G2/M phase.
**Weiner, David B.**, Merion, PA, United States
Ayyavoo, Velpandi, Havertown, PA, United States
Mahalingam, Sundarasamy, Birmingham, AL, United States
Patel, Mamata, Philadelphia, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 6172201 B1 20010109
APPLICATION: US 1999-418175 19991013 (9)
PRIORITY: US 1997-55754P 19970814 (60)
DOCUMENT TYPE: Patent; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to substantially pure human **Vpr**

[illegible] isolated nucleic acid molecules that encode hVIP, or a fragment thereof; nucleic acid probes and primers directed to nucleic acid molecules that encode hVIP, or a fragment thereof; oligonucleotide molecules that consist of a nucleotide sequence complementary to a portion of the nucleotide sequence that encodes hVIP; vectors comprising nucleic acid molecules encoding hVIP; recombinant expression vectors that comprise nucleic acid sequences that encode hVIP; host cells that comprise recombinant expression vectors which include nucleic acid sequences that encode hVIP; genetic therapy vectors comprising nucleic acid molecules encoding hVIP; isolated **antibody** which binds to an epitope on hVIP; pharmaceutical compositions comprising a pharmaceutically acceptable carrier and nucleic acid molecules complementary to a portion of hVIP; methods of making hVIP; and methods of inhibiting expression of hVIP oligonucleotides complementary to a portion of the nucleotide sequence that encodes hVIP.

CLM What is claimed is:

1. An isolated **antibody** which binds to an epitope on a protein having the amino acid sequence of SEQ ID NO:2.

2. The **antibody** of claim 1 wherein said **antibody** is a monoclonal **antibody**.

3. The **antibody** of claim 1 wherein the **antibody** is an **antibody** fragment.

4. The **antibody** of claim 3 wherein the **antibody** fragment is a Fab fragment.

5. The **antibody** of claim 3 wherein the **antibody** fragment is a $F(ab)_2$ fragment.

6. A hybridoma cell line which produces **antibodies** according to claim 1.

L4 ANSWER 13 OF 26 USPATFULL on STN

2000:88313 Nucleotide sequences encoding **vpr** receptor protein.

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Ayyavoo, Velpandi, Havertown, PA, United States
Mahalingham, Sundarasama, Philadelphia, PA, United States
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US 6087486 20000711
APPLICATION: US 1998-14877 19980128 (9)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB DNA molecules which encode rip-1 protein sequences are disclosed. Expression vectors and host cells which include the DNA molecules are disclosed.

CLM What is claimed is:

1. A DNA molecule having a nucleotide sequence consisting of less than 4000 nucleotides and comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, a fragment of SEQ ID NO:6 having at least 100 nucleotides, a fragment of SEQ ID NO:7 having at least 100 nucleotides, and a fragment of SEO ID NO:8 having at least 100 nucleotides.

2. The DNA molecule of claim 1 consisting of up to 1000 nucleotides.

3. A DNA molecule having a nucleotide sequence consisting of less then 4000 nucleotides and comprising SEQ ID NO:6.

4. A DNA molecule having a nucleotide sequence consisting of less then 4000 nucleotides and comprising SEQ ID NO:7.

5. A DNA molecule having a nucleotide sequence consisting of less then

1000 nucleotides and comprising SEQ ID NO:6.

6. The DNA molecule of claim 1 consisting of a nucleotide sequence selected from the group consisting of: SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

7. A recombinant vector comprising a nucleotide sequence comprising one or more of: SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

8. The recombinant vector of claim 7 comprising SEQ ID NO:6.

9. The recombinant vector of claim 7 comprising SEQ ID NO:7.

10. The recombinant vector of claim 7 comprising SEQ ID NO:8.

11. The recombinant vector of claim 7 comprising SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

12. A recombinant host cell comprising a recombinant vector according to claim 7.

13. The recombinant host cell of claim 12 wherein said vector comprises SEQ ID NO:6.

14. The recombinant host cell of claim 12 wherein said vector comprises SEQ ID NO:7.

15. The recombinant host cell of claim 12 wherein said vector comprises SEQ ID NO:8.

16. The recombinant host cell of claim 12 wherein said vector comprises SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

17. The DNA molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8.

18. The DNA molecule of claim 6 consisting of SEQ ID NO:6.

19. The DNA molecule of claim 6 consisting of SEQ ID NO:7.

20. The DNA molecule of claim 6 consisting of SEQ ID NO:8.

L4 ANSWER 14 OF 26 USPATFULL on STN
2000:57881 Cellular receptor for HIV-1 **VPR** essential for G2/M phase transition of the cell cycle.
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Ayyavoo, Velpandi, Havertown, PA, United States
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The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 6060587 20000509
APPLICATION: US 1997-949202 19971010 (8)
PRIORITY: US 1997-55754P 19970814 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed to substantially pure human **Vpr** Interacting Protein (hVIP), and fragments thereof. Also disclosed are isolated nucleic acid molecules that encode hVIP, or a fragment thereof; nucleic acid probes and primers directed to nucleic acid molecules that encode hVIP, or a fragment thereof; oligonucleotide molecules that consist of a nucleotide sequence complementary to a portion of the nucleotide sequence that encodes hVIP; vectors comprising nucleic acid molecules encoding hVIP; recombinant expression vectors that comprise

nucleic acid sequences that encode hVIP; host cells that comprise recombinant expression vectors which include nucleic acid sequences that encode hVIP; genetic therapy vectors comprising nucleic acid molecules encoding hVIP; isolated **antibody** which binds to an epitope on hVIP; pharmaceutical compositions comprising a pharmaceutically acceptable carrier and nucleic acid molecules complementary to a portion of hVIP; methods of making hVIP; and methods of inhibiting expression of hVIP oligonucleotides complementary to a portion of the nucleotide sequence that encodes hVIP.

CLM What is claimed is:

1. A substantially pure protein having the amino acid sequence of SEQ ID NO:2.

2. A recombinant expression vector comprising a nucleic acid sequence that encodes the protein of claim 1.

3. A host cell comprising the recombinant expression vector of claim 2.

4. The host cell of claim 3 comprising a recombinant expression vector that comprises SEQ ID NO:1.

5. An isolated nucleic acid molecule consisting of SEQ ID NO:1, or a fragment thereof having at least 10 nucleotides.

6. The nucleic acid molecule of claim 5 consisting of a fragment of SEQ ID NO:1 having at least 10 nucleotides.

7. The nucleic acid molecule of claim 5 consisting of a fragment of SEQ ID NO:1 having 12-150 nucleotides.

8. The nucleic acid molecule of claim 5 consisting of a fragment of SEQ ID NO:1 having 15-50 nucleotides.

9. The nucleic acid molecule of claim 5 consisting of a fragment of SEQ ID NO:1 having 18-30 nucleotides.

10. The nucleic acid molecule of claim 5 consisting of a fragment of SEQ ID NO:1 having 24 nucleotides.

11. An oligonucleotide molecule comprising a nucleotide sequence complementary to a nucleotide sequence of at least 10 nucleotides of SEQ ID NO:1.

12. The oligonucleotide molecule of claim 11 consisting of a nucleotide sequence complementary to a nucleotide sequence of 10-150 nucleotides of SEQ ID NO:1.

13. The oligonucleotide molecule of claim 11 consisting of a nucleotide sequence complementary to a nucleotide sequence of 18-28 nucleotides of SEQ ID NO:1.

14. A pharmaceutical composition comprising: a) a nucleic acid molecule that comprises SEQ ID NO: 1 or a fragment from the coding region thereof having at least 100 nucleotides; and b) a pharmaceutically acceptable carrier.

15. A pharmaceutical composition comprising: a) an oligonucleotide that comprises a nucleotide sequence complementary to a nucleotide sequence of at least 10 nucleotides of the coding region of SEQ ID NO: 1; and b) a pharmaceutically acceptable carrier.

16. A method of making human **Vpr** Interacting Protein comprising: isolating nucleic acid molecule having SEQ ID NO:1; inserting said nucleic acid molecule into an expression vector; inserting said expression vector into host cell under conditions in which said protein is expressed; and isolating said human **Vpr** Interacting Protein.

17. A plasmid comprising a nucleic acid sequence that encodes a protein that has the amino acid sequence of SEQ ID NO:2.

18. The plasmid of claim 17 comprising SEQ ID NO:1.

19. An isolated nucleic acid molecule consisting of SEQ ID NO:1.

20. A recombinant expression vector comprising the nucleic acid molecule of claim 19.

21. A host cell comprising the recombinant expression vector of claim 20.

22. An isolated nucleic acid molecule consisting of a fragment of the coding region of SEQ ID NO: 1 having at least 10 contiguous nucleotides of SEQ ID NO: 1.

23. An isolated nucleic acid molecule consisting of a fragment of the coding region of SEQ ID NO: 1 having 12-150 contiguous nucleotides of SEQ ID NO: 1.

24. An isolated nucleic acid molecule consisting of a fragment of the coding region of SEQ ID NO: 1 having 15-50 contiguous nucleotides of SEQ ID NO: 1.

25. An isolated nucleic acid molecule consisting of a fragment of the coding region of SEQ ID NO: 1 having 18-30 contiguous nucleotides of SEQ ID NO: 1.

26. An isolated nucleic acid molecule consisting of a fragment of the coding region of SEQ ID NO: 1 having 24 contiguous nucleotides of SEQ ID NO: 1.

27. An isolated nucleic acid molecule comprising a fragment of SEQ ID NO: 1 having 100 contiguous nucleotides of SEQ ID NO: 1.

28. The isolated nucleic acid molecule of claim 27 comprising a fragment of the coding region of SEQ ID NO: 1 having 100 contiguous nucleotides of SEQ ID NO: 1.

29. An isolated nucleic acid molecule comprising a fragment of SEQ ID NO: 1 having 150 contiguous nucleotides of SEQ ID NO: 1.

30. The isolated nucleic acid molecule of claim 29 comprising a fragment of the coding region of SEQ ID NO: 1 having 150 contiguous nucleotides of SEQ ID NO: 1.

31. The pharmaceutical composition of claim 14 wherein said nucleic acid molecule comprises a fragment of SEQ ID NO:1 having at least 150 contiguous nucleotides of SEQ ID NO:1.

32. A pharmaceutical composition comprising: a) a nucleic acid molecule that consists of SEQ ID NO: 1 or a fragment of SEQ ID NO:1 having at least 10 contiguous nucleotides of SEQ ID NO:1; and b) a pharmaceutically acceptable carrier.

33. The pharmaceutical composition of claim 32 wherein said fragment of SEQ ID NO:1 has 12-150 contiguous nucleotides of SEQ ID NO:1.

34. The pharmaceutical composition of claim 14 wherein said fragment of SEQ ID NO:1 has 15-50 contiguous nucleotides of SEQ ID NO:1.

35. The pharmaceutical composition of claim 14 wherein said fragment of SEQ ID NO:1 has 18-30 contiguous nucleotides of SEQ ID NO:1.

L4 ANSWER 15 OF 26 USPATFULL on STN
1999:141912 Compositions and methods for delivery of genetic material.
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US 5981505 19991109
WO 9416737 19940804
APPLICATION: US 1997-979385 19971126 (8)
WO 1994-US899 19940126 19950828 PCT 371 date 19950828 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of inducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a polynucleotide function enhancer and administering to the cells, a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produces a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:

1. A pharmaceutical composition comprising: a) a polynucleotide function enhancer; and b) a DNA molecule that comprises a DNA sequence that encodes an antigen; wherein said polynucleotide function enhancer is selected from the group consisting of bupivacaine and tetracaine and said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

2. The pharmaceutical composition of claim 1 wherein said composition comprises bupivacaine.

3. The pharmaceutical composition of claim 1 wherein said composition comprises tetracaine.

4. The pharmaceutical composition of claim 1 wherein said DNA molecule is a plasmid.

5. The pharmaceutical composition of claim 1 wherein said DNA sequence encodes a variable region of a T cell receptor.

6. The pharmaceutical composition of claim 1 wherein said DNA sequence encodes a pathogen antigen.

7. The pharmaceutical composition of claim 6 wherein said DNA sequence encodes an antigen from an intracellular pathogen.

8. The pharmaceutical composition of claim 7 wherein said antigen is a viral antigen.

9. The pharmaceutical composition of claim 7 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

10. The pharmaceutical composition of claim 9 wherein said pathogen is Herpes simplex 2 virus, HSV2.

11. The pharmaceutical composition of claim 9 wherein said pathogen is Hepatitis B virus, HBV.

12. The pharmaceutical composition of claim 9 wherein said pathogen is human T cell leukemia virus, HTLV.

13. The pharmaceutical composition of claim 9 wherein said pathogen is a human immunodeficiency virus.

14. The pharmaceutical composition of claim 1 wherein said DNA sequence encodes a hyperproliferative disease associated protein.

15. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is cancer.

16. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a lymphoma.

17. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a melanoma.

18. A method of immunizing an individual comprising the steps of: injecting into tissue of said individual at a site on said individual's body, a DNA molecule and a polynucleotide function enhancer, said DNA molecule comprising a DNA sequence that encodes an antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence, said polynucleotide function enhancer selected from the group consisting of bupivacaine and tetracaine; wherein said DNA molecule is taken up by cells in said tissue, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

19. The method of claim 18 wherein said tissue includes skin and skeletal muscle.

20. The method of claim 18 wherein said tissue is skin.

21. The method of claim 18 wherein said tissue is muscle.

22. The method of claim 18 wherein said tissue is skeletal muscle.

23. The method of claim 18 wherein said polynucleotide function enhancer is bupivacaine.

24. The method of claim 18 wherein said polynucleotide function enhancer is tetracaine.

25. The method of claim 18 wherein said DNA molecule is a plasmid.

26. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

27. The method of claim 26 wherein said pathogen is an intracellular pathogen.

28. The method of claim 27 wherein said intracellular pathogen is a virus.

29. The method of claim 26 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis a virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1

virus; HSV1; Herpes simplex 2 virus; HSV2; Cytomegalovirus; CMV; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

30. The method of claim 18 wherein said immune response generated against said antigen provides a protective immune response against a pathogen and said individual is immunized against said pathogen.

31. The method of claim 30 wherein said tissue is skin.

32. The method of claim 30 wherein said tissue is skeletal muscle.

33. The method of claim 30 wherein said polynucleotide function enhancer is bupivacaine.

34. The method of claim 30 wherein said polynucleotide function enhancer is tetracaine.

35. The method of claim 30 wherein said DNA molecule is a plasmid.

36. The method of claim 30 wherein said antigen is a pathogen antigen.

37. The method of claim 30 wherein said pathogen is an intracellular pathogen.

38. The method of claim 37 wherein said intracellular pathogen is a virus.

39. The method of claim 38 wherein said virus is selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

40. The method of claim 39 wherein said virus is Herpes simplex 2 virus, HSV2.

41. The method of claim 39 wherein said virus is Hepatitis B virus, HBV.

42. The method of claim 39 wherein said virus is human T cell leukemia virus, HTLV.

43. The method of claim 18 wherein said immune response generated against said antigen provides a therapeutic immune response against a pathogen in an individual who is infected with said pathogen.

44. The method of claim 43 wherein said tissue is skin.

45. The method of claim 43 wherein said tissue is skeletal muscle.

46. The method of claim 43 wherein said polynucleotide function enhancer is bupivacaine.

47. The method of claim 43 wherein said polynucleotide function enhancer is tetracaine.

48. The method of claim 43 wherein said DNA molecule is a plasmid.

49. The method of claim 43 wherein said pathogen is an intracellular pathogen.

50. The method of claim 43 wherein said pathogen is a virus.

51. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a hyperproliferative disease-associated protein.

52. The method of claim 51 wherein said tissue is skin.

53. The method of claim 51 wherein said tissue is skeletal muscle.

54. The method of claim 51 wherein said polynucleotide function enhancer is bupivacaine.

55. The method of claim 51 wherein said polynucleotide function enhancer is tetracaine.

56. The method of claim 51 wherein said DNA molecule is a plasmid.

57. The method of claim 51 wherein said hyperproliferative disease-associated protein is selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, src, neu and trk; protein products of translocation gene bcr/abl; P53; variable regions of **antibodies** made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

58. The method of claim 51 wherein said immune response generated against said antigen is a therapeutically effective immune response against a hyperproliferative disease-associated protein in an individual who has a hyperproliferative disease.

59. The method of claim 58 wherein said hyperproliferative disease is cancer.

60. The method of claim 58 wherein said hyperproliferative disease is a melanoma.

61. The method of claim 58 wherein said hyperproliferative disease is a lymphoma.

62. The method of claim 18 wherein said immune response generated against said antigen is an immune response against an autoimmune disease-associated protein.

63. The method of claim 62 wherein said tissue is skin.

64. The method of claim 62 wherein said tissue is skeletal muscle.

65. The method of claim 62 wherein said polynucleotide function enhancer is bupivacaine.

66. The method of claim 62 wherein said polynucleotide function enhancer is tetracaine.

67. The method of claim 62 wherein said DNA molecule is a plasmid.

68. The method of claim 62 wherein said autoimmune disease associated-protein is selected from the group consisting of: variable regions of **antibodies** involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

69. A method of introducing DNA molecules into cells of an individual comprising the step of: injecting into tissue of said individual at a site on said individual's body, DNA molecules and a polynucleotide function enhancer selected from the group consisting of bupivacaine and tetracaine, wherein said DNA molecules are taken up by cells in said tissue.

70. The method of claim 69 wherein said DNA molecule comprises a DNA sequence that encodes an protein, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA

sequence.

71. The method of claim 69 wherein said tissue is skin.

72. The method of claim 69 wherein said tissue is skeletal muscle.

73. The method of claim 69 wherein said polynucleotide function enhancer is bupivacaine.

74. The method of claim 69 wherein said polynucleotide function enhancer is tetracaine.

75. The method of claim 69 wherein said DNA molecule is a plasmid.

L4 ANSWER 16 OF 26 USPATFULL on STN
1999:121330 Compositions and methods for delivery of genetic material.
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US 5962428 19991005
WO 9526718 19951012
APPLICATION: US 1996-704701 19960916 (8)
WO 1995-US4071 19950330 19960916 PCT 371 date 19960916 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of introducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a genetic vaccine facilitator and administering to the cells a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produces a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.
CLM What is claimed is:
1. A method of generating an immune response in an individual against an antigen comprising administering in vivo to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

2. The method of claim 1 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: salts of lauric and oleic acids, lauric and oleic acids, acid esters of lauryl and cetyl alcohol, and sulfonates.

3. The method of claim 1 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: sodium lauryl sulfate and oleic acid.

4. The method of claim 1 wherein said genetic vaccine facilitator is a

saponin selected from the group consisting of: saponarin, sarmentocymarin and sapogenins.

5. The method of claim 1 wherein said genetic vaccine facilitator is a saponin selected from the group consisting of: sarmentogenin, sarsasapogenin and sarverogenin.

6. The method of claim 1 wherein said genetic vaccine facilitator is a lectin selected from the group consisting of: concanavalin A, abrin, soybean agglutinin and wheat germ agglutinin.

7. The method of claim 1 wherein said genetic vaccine facilitator is concanavalin A.

8. The method of claim 1 wherein said genetic vaccine facilitator is β-estradiol.

9. The method of claim 1 wherein said genetic vaccine facilitator is selected from the group consisting of: ethanol, n-propanol, isopropanol and n-butanol.

10. The method of claim 1 wherein said genetic vaccine facilitator is dimethyl sulfoxide.

11. The method of claim 1 wherein said genetic vaccine facilitator is urea.

12. A method of generating an immune response in an individual against a pathogen comprising administering in vivo to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, said nucleotide sequence being operably linked to regulatory sequences; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

13. The method of claim 12 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

14. The method of claim 12 wherein said DNA molecule is a plasmid.

15. The method of claim 12 wherein said protein is a pathogen antigen or a fragment thereof which is antigenic.

16. The method of claim 12 wherein said DNA molecule is administered intramuscularly.

17. The method of claim 12 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus, HAV; hepatitis B virus, HBV; hepatitis C virus, HCV; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBV; rhinovirus; and, coronavirus.

18. The method of claim 12 wherein at least two or more different nucleic acid molecules are administered to different cells of an individual; said different nucleic acid molecules each comprising DNA sequences encoding one or more pathogen antigens of the same pathogen.

19. The method of claim 12 wherein said genetic vaccine facilitator and said DNA molecule are administered simultaneously.

20. A method of generating an immune response in an individual against a disease comprising administering in vivo to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes a protein which comprises an epitope identical or substantially similar to an epitope of a protein associated with said disease operatively linked to regulatory sequences; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said target protein.

21. The method of claim 20 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

22. The method of claim 20 wherein said disease is characterized by hyperproliferating cells.

23. The method of claim 20 wherein said disease is an autoimmune disease.

24. The method of claim 20 wherein said DNA molecule is a plasmid.

25. The method of claim 20 wherein said DNA molecule is administered intramuscularly.

26. The method of claim 20 wherein said DNA molecule comprises a DNA sequence that encodes a protein selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, sarc, neu and trk; protein products of translocation gene bcl/abl; p53; EGRF; variable regions of **antibodies** made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

27. The method of claim 20 wherein said protein is selected from the group consisting of: variable regions of **antibodies** involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

28. A pharmaceutical composition comprising: i) a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence which encodes a protein wherein said DNA sequence is operably linked to regulatory sequences required for expression in a mammal and said protein is selected from the group consisting of: proteins which comprise at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen; proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with hyperproliferating cells; and proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with an autoimmune disease; and ii) a genetic vaccine facilitator selected from the group consisting of anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea.

29. The pharmaceutical composition of claim 28 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

30. A pharmaceutical kit comprising: i) a container that comprises a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence which encodes a protein wherein said DNA sequence is operably linked to regulatory sequences required for expression in a mammal and said protein is selected from the group consisting of: proteins which comprise at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen; proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with hyperproliferating cells; and proteins which comprise an epitope identical or substantially similar to an epitope of a protein associated with an autoimmune disease; and ii) a container that comprises a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea.

31. The pharmaceutical kit of claim 30 wherein said genetic vaccine facilitator is selected from the group consisting of: sodium lauryl sulfate; oleic acid; saponarin; sarmentocymarin; sapogenins; sarmentogenin; sarsasapogenin; sarverogenin; concanavalin A; β-estradiol; ethanol; dimethyl sulfoxide; and urea.

32. A method of delivering a protein into cells of an individual in vivo comprising administering to muscle or skin of said individual's body a genetic vaccine facilitator selected from the group consisting of: anionic lipids; saponins; lectins; estrogenic compounds; hydroxylated lower alkyls; dimethyl sulfoxide; and urea; and, a DNA molecule that is dissociated from an infectious agent and comprises a DNA sequence that encodes said protein, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells producing said protein in said cells.

33. The method of claim 30 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: salts of lauric and oleic acids, lauric and oleic acids, acid esters of lauryl and cetyl alcohol, and sulfonates.

34. The method of claim 30 wherein said genetic vaccine facilitator is an anionic lipid selected from the group consisting of: sodium lauryl sulfate and oleic acid.

35. The method of claim 30 wherein said genetic vaccine facilitator is a saponin selected from the group consisting of: saponarin, sarmentocymarin and sapogenins.

36. The method of claim 30 wherein said genetic vaccine facilitator is a saponin selected from the group consisting of: sarmentogenin, sarsasapogenin and sarverogenin.

37. The method of claim 30 wherein said genetic vaccine facilitator is a lectin selected from the group consisting of: concanavalin A, abrin, soybean agglutinin and wheat germ agglutinin.

38. The method of claim 30 wherein said genetic vaccine facilitator is concanavalin A.

39. The method of claim 30 wherein said genetic vaccine facilitator is β-estradiol.

40. The method of claim 30 wherein said genetic vaccine facilitator is selected from the group consisting of: ethanol, n-propanol, isopropanol and n-butanol.

41. The method of claim 30 wherein said genetic vaccine facilitator is dimethyl sulfoxide.

12. The method of claim 9 wherein said genetic vaccine facilitator is urea.

L4 ANSWER 17 OF 26 USPATFULL on STN
1999:24450 Identification of compounds that modulate HIV-1 **vpr** protein activity

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Trustees of The University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 5874225 19990223
APPLICATION: US 1993-19601 19930219 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed toward methods of identifying compounds which inhibit the human immunodeficiency virus (HIV) **viral protein R** (**Vpr**) from stimulating the differentiation of undifferentiated cells. This invention takes advantage of the observation that cell lines from rhabdomyosarcomas, which are tumors of muscle origin, have been used as models of CD4-independent HIV infection. These cell lines can be induced to differentiate in vitro. The **vpr** gene of HIV-1 is sufficient for the differentiation of the human rhabdomyosarcoma cell line TE671. Differentiated cells are characterized by great enlargement, altered morphology, lack of replication, and high level expression of the muscle-specific protein myosin. Morphological differentiation and inhibition of proliferation of other transformed cell lines following **vpr** expression was also observed. This invention also relates toward methods of identifying compounds which inhibit HIV **Vpr** binding to Gag. These screening methods should facilitate the identification and development of antiviral agents.
CLM What is claimed is:
1. An in vitro method of identifying compounds that are capable of inhibiting HIV-1 **Vpr**-mediated differentiation of undifferentiated cells comprising the following steps: (I) contacting undifferentiated cells with HIV-1 **Vpr** in the presence or absence of a test compound; and, (ii) determining whether said cells cease proliferating and display cellular differentiation markers in the presence or absence of a test compound; wherein the presence of cellular proliferation and absence of differentiation markers in the test sample is indicative of said compound being capable of inhibiting HIV-1 **Vpr**-mediated differentiation of undifferentiated cells.

2. The method of claim 1 wherein said undifferentiated cells are selected from the group consisting of: solid muscle tumor alveolar rhabdomyosarcoma cell line RD, solid muscle tumor alveolar rhabdomyosarcoma cell line TE671, osteosarcoma cell line D17, osteosarcoma cell line MG63, osteosarcoma cell line HOS-TE86, myeloid lineage cell line KG-1, myeloid lineage cell line THP-1, myeloid lineage cell line PLB973, human glioblastoma cell line U-138MG, human glioblastoma/astrocytoma cell line U373MG, and human glioblastoma/astrocytoma cell line U87-MG.

3. An in vitro method of identifying compounds that are capable of inhibiting HIV-1 **Vpr**-mediated suppression of cellular proliferation comprising the following steps: (I) contacting proliferating cells with HIV-1 **Vpr** in the presence or absence of a test compound; and, (ii) determining whether said cells cease proliferating in the presence or absence of said test compound; wherein the presence of cellular proliferation in the test sample is indicative of said compound being capable of inhibiting HIV-1 **Vpr**-mediated suppression of cellular proliferation.

4. The method of claim 3 wherein said proliferating cells are selected

from the group consisting of: solid muscle tumor alveolar rhabdomyosarcoma cell line RD, solid muscle tumor alveolar rhabdomyosarcoma cell line TE671, osteosarcoma cell line D17, osteosarcoma cell line MG63, osteosarcoma cell line HOS-TE86, myeloid lineage cell line KG-1, myeloid lineage cell line THP-1, myeloid lineage cell line PLB973, human glioblastoma cell line U-138MG, human glioblastoma/astrocytoma cell line U373MG, and human glioblastoma/astrocytoma cell line U87-MG.

5. An in vitro method for the identification of compounds capable of inhibiting HIV-1 **Vpr** binding to HIV-1 Gag comprising the following steps: (I) contacting, in the presence or absence of a test compound, HIV-1 **Vpr** and Gag; and (ii) determining the level of binding between HIV-1 **Vpr** and Gag, wherein a reduction in binding in the presence of the test compound is indicative of said compound being capable of inhibiting HIV-1 **Vpr** binding to Gag.

6. The method of claim 5 wherein said binding level is determined by the addition of a labeled **antibody**.

7. The method of claim 5 wherein said HIV-1 **Vpr** and Gag are produced in eukaryotic cells.

8. The method of claim 5 wherein said HIV-1 **Vpr** and Gag are produced in insect cells.

9. The method of claim 5 comprising the following steps: (I) contacting, in the presence or absence of a test compound, eukaryotically expressed HIV-1 **Vpr** and Gag, wherein said **Vpr** is attached to a solid support; (ii) washing the mixture of step (I) to remove unbound Gag protein; and, (iii) determining the level of binding between HIV-1 **Vpr** and Gag through the addition of a Gag-specific labeled **antibody**, wherein a reduction in binding in the presence of the test compound is indicative of said compound being capable of inhibiting HIV-1 **Vpr** binding to Gag.

10. The method of claim 9 wherein said HIV-1 **Vpr** and Gag are produced in insect cells.

11. The method of claim 5 comprising the following steps: (I) contacting, in the presence or absence of a test compound, eukaryotically expressed HIV-1 **Vpr** and Gag, wherein said Gag is attached to a solid support; (ii) washing the mixture of step (I) to remove unbound **Vpr** protein; and, (iii) determining the level of binding between HIV-1 **Vpr** and Gag through the addition of a **Vpr**-specific labeled **antibody**, wherein a reduction in binding in the presence of the test compound is indicative of said compound being capable of inhibiting HIV-1 **Vpr** binding to Gag.

12. The method of claim 11 wherein said HIV-1 **Vpr** and Gag are produced in insect cells.

L4 ANSWER 18 OF 26 USPATFULL on STN
1998:135023 Genetic immunization.
**Weiner, David B.**, Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 5830876 19981103
APPLICATION: US 1995-453349 19950530 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method of immunizing an individual against pathogen is disclosed. Also disclosed is a method of treating an individual who has a

hyperproliferative disease, or of treating an individual who is infected by a pathogen. Specifically, the individual is injected with bupivacaine along with DNA in an expressible form, the DNA encoding an antigen. The encoded antigen can be from a protein from the pathogen or from a protein associated with the hyperproliferative disease.

CLM What is claimed is:

1. A method of immunizing an individual against a pathogen comprising the steps of: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes an antigen from said pathogen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells and a protective immune response is generated against said antigen.

2. The method of claim 1 wherein said pathogen is an intracellular pathogen.

3. The method of claim 1 wherein said pathogen is a virus.

4. The method of claim 1 wherein said pathogen is a virus selected from the group consisting of: human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

5. The method of claim 1 wherein said pathogen is Herpes simplex 2 virus, HSV2.

6. The method of claim 1 wherein said pathogen is Hepatitis B virus, HBV.

7. The method of claim 1 wherein said pathogen is human T cell leukemia virus, HTLV.

8. A method of treating an individual who has a hyperproliferative disease comprising: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes a hyperproliferative disease-associated protein operatively linked to regulatory sequences; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells, and a therapeutically effective immune response is generated against said hyperproliferative disease-associated protein, said immune response being directed at hyperproliferating cells expressing said hyperproliferative disease-associated protein.

9. The method of claim 8 wherein said hyperproliferative disease is cancer.

10. The method of claim 8 wherein said hyperproliferative disease is a lymphoma.

11. The method of claim 8 wherein said hyperproliferative disease is T cell lymphoma and said hyperproliferative disease-associated protein is a T cell antigen.

12. The method of claim 8 wherein said hyperproliferative disease is T cell lymphoma and said DNA sequence encodes a variable region of a T cell receptor.

13. The method of claim 8 wherein said hyperproliferative disease is a melanoma.

14. A method of treating an individual who is infected by a pathogen comprising: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes an antigen from said pathogen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells and a therapeutically effective immune response is generated.

15. The method of claim 14 wherein said pathogen is an intracellular pathogen.

16. The method of claim 14 wherein said pathogen is a virus.

17. The method of claim 14 wherein said pathogen is human immunodeficiency virus HIV.

L4 ANSWER 19 OF 26 USPATFULL on STN
1998:122388 Genetic immunization.
**Weiner, David B.**, Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)
US 5817637 19981006
APPLICATION: US 1997-783818 19970113 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:
1. An pharmaceutical immunizing kit comprising: a) a first inoculant comprising: i) a pharmaceutically acceptable carrier or diluent; and, ii) a first nucleic acid molecule comprising a nucleotide sequence that encodes at least one HIV protein operatively linked to regulatory sequences; wherein said nucleotide sequence is capable of being expressed in human cells; b) a second inoculant comprising: i) a pharmaceutically acceptable carrier or diluent; and, ii) a second nucleic acid molecule comprising a nucleotide sequence that encodes at least one HIV protein operatively linked to regulatory sequences; wherein said nucleotide sequence is capable of being expressed in human cells; wherein said first nucleic acid molecule is not identical to said second nucleic acid molecule and, taken together, said first nucleic acid molecule and said second nucleic acid molecule encode HIV proteins gag, pol and env; and c) a third inoculant comprising bupivacaine.

2. A pharmaceutical composition comprising: a) a compound selected from the group consisting of: bupivacaine, mepivacaine, lidocaine, procaine, carbocaine and methyl bupivacaine; and b) a DNA molecule that comprises a DNA sequence that encodes an antigen; wherein said DNA sequence

operatively linked to regulatory sequences which control the expression of said DNA sequence.

3. The pharmaceutical composition of claim 2 wherein said composition comprises bupivacaine.

4. The pharmaceutical composition of claim 2 wherein said DNA molecule is a plasmid.

5. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a variable region of a T cell receptor.

6. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a pathogen antigen.

7. The pharmaceutical composition of claim 6 wherein said DNA sequence encodes an antigen from an intracellular pathogen.

8. The pharmaceutical composition of claim 7 wherein said antigen is a viral antigen.

9. The pharmaceutical composition of claim 8 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

10. The pharmaceutical composition of claim 9 wherein said pathogen is Herpes simplex 2 virus, HSV2.

11. The pharmaceutical composition of claim 9 wherein said pathogen is Hepatitis B virus, HBV.

12. The pharmaceutical composition of claim 9 wherein said pathogen is human T cell leukemia virus, HTLV.

13. The pharmaceutical composition of claim 9 wherein said pathogen is a human immunodeficiency virus.

14. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a hyperproliferative disease associated protein.

15. The pharmaceutical composition of claim 13 wherein said hyperproliferative disease is cancer.

16. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a lymphoma.

17. The pharmaceutical composition of claim 14 wherein said hyperproliferative disease is a melanoma.

18. a method of immunizing an individual against an antigen comprising administering to tissue of said individual's body, a) a compound selected from the group consisting of bupivacaine, mepivacaine, lidocaine, procaine, carbocaine and methyl bupivacaine, and b) a DNA molecule that comprises a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

19. The method of claim 18 wherein said compound is bupivacaine.

20. The method of claim 18 wherein said DNA molecule is a plasmid.

21. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

22. The method of claim 21 wherein said pathogen is an intracellular pathogen.

23. The method of claim 22 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis a virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV; Epstein-Barr virus, EBR; rhinovirus; and, coronavirus.

24. The method of claim 23 wherein said pathogen is HIV and said DNA molecule comprises a DNA sequence that encodes an HIV antigen.

25. The method of claim 18 wherein said immune response generated against said antigen is an immune response against a hyperproliferative disease-associated protein.

26. The method of claim 25 wherein said hyperproliferative disease-associated protein is selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, src, neu and trk; protein products of translocation gene bcr/abl; P53; variable regions of **antibodies** made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

27. The method of claim 18 wherein said immune response generated against said antigen is an immune response against an autoimmune disease-associated protein.

28. The method of claim 27 wherein said autoimmune disease-associated protein is selected from the group consisting of: variable regions of **antibodies** involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

29. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered subcutaneously.

30. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered intramuscularly, intraperitoneally, intravenously, intraarterially, intraoccularly, orally transdermally and/or by inhalation.

31. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered intradermally.

32. The method of claim 31 wherein said immune response generated against said antigen is an immune response against a pathogen antigen.

33. The method of claim 31 wherein said immune response generated against said antigen is an immune response against a hyperproliferative disease-associated protein.

34. The method of claim 31 wherein said immune response generated against said antigen is an immune response against an autoimmune disease-associated protein.

L4 ANSWER 20 OF 26 USPATFULL on STN
1998:82540 **VPR** receptor protein.
**Weiner, David B.**, Merion, PA, United States
Levy, David Nathan, Boston, MA, United States
Refaeli, Yosef, Boston, MA, United States

The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5780238 19980714
WO 9516705 19950622
APPLICATION: US 1996-652572 19961024 (8)
WO 1994-US14532 19941215.19961024 PCT 371 date 19961024 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A human receptor protein which binds to the human immunodeficiency virus (HIV) **viral protein R** (**vpr**) is disclosed. Pharmaceutical compositions that comprise the receptor protein, compositions useful to produce the receptor protein and methods of making and using the receptor protein are disclosed.

CLM What is claimed is:

1. Essentially pure **viral protein R** receptor protein characterized by a molecular weight of about 41 kD as determined using 12% SDS-PAGE, an ability to bind to **viral protein R** and solubility in Triton, or a fragment of said **viral protein R** receptor protein which binds to **viral protein R**.

2. The protein of claim 1 wherein said protein is characterized by a molecular weight of about 41 kD as determined using 12% SDS-PAGE, an ability to bind to **viral protein R** and solubility in Triton.

3. The protein of claim 1 wherein said protein is a fragment of the protein which has a molecular weight of about 41 kD as determined using 12% SDS-PAGE, said fragment having the ability to bind to **viral protein R** and solubility in Triton.

4. A method of identifying compounds which inhibit binding of **viral protein R** to the viral protein receptor protein of claim 1 which comprises the steps of: a) contacting in the presence of a test compound, **viral protein R** protein or a fragment thereof and said **viral protein R** receptor protein or a fragment thereof, wherein in the absence of said test compound said **viral protein R** protein or said fragment thereof binds to said **viral protein R** receptor protein or said fragment thereof; b) determining the level of binding and c) comparing that level to the level of binding that occurs when **viral protein R** protein and said **viral protein R** receptor protein are contacted in the absence of a test compound, wherein a decrease in binding levels in the presence of said test compound indicates that the test compound is a compound which inhibits binding of **viral protein R** protein to the **viral protein R** receptor protein of claim 1.

5. A kit for identifying compounds which inhibit binding of **viral protein R** protein to the **viral protein R** receptor protein of claim 1 which comprises a) a first container which contains **viral protein R** protein or a fragment thereof which binds **viral protein R** receptor protein or a fragment thereof, and b) a second container which contains said **viral protein R** receptor protein or a fragment thereof which binds **viral protein R** protein or a fragment thereof.

L4 ANSWER 21 OF 26 USPATFULL on STN
1998:82523 Methods and compositions for inhibiting HIV replication.
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Levy, David N., Birmingham, AL, United States
Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5780220 19980714
APPLICATION: US 1995-382873 19950203 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for treating an individual exposed to or infected with HIV is disclosed which comprises administering to said individual a therapeutically effective amount of one or more compounds which inhibit or prevent replication of said HIV by interfering with the replicative or other essential functions of **Vpr** expressed by said HIV, by interactively blocking the **Vpr** target in human cells, and thereby preventing translocation of the **Vpr**/target complex from the cytosol of said human cells to the nuclei of said cells, where **Vpr** carries on activities essential to replication of HIV. In preferred embodiments, the compound or compounds which interactively block the target are steroid hormone receptor antagonists, glucocorticoid receptor antagonists, or glucocorticoid receptor Type II antagonists, especially mifepristone (RU-486). Pharmaceutical compositions comprising these compounds, as well as a method for identifying them and a kit for use therein, are also disclosed.

CLM What is claimed is:

1. A method for treating a human individual exposed to or infected with HIV comprising the steps of identifying said individual, and administering to said individual a therapeutically effective amount of mifepristone to inhibit or prevent replication of said HIV by inhibiting cytosolic-nuclear translocation of a complex comprising HIV **Vpr** protein and Rip-1 protein in an HIV infected cell of said individual.

2. A method according to claim 1 further comprising coadministering to said individual one or more therapeutic agents useful for treating HIV infected individuals, selected from the group consisting of zidovudine (AZT), acyclovir, ganciclovir, foscarnet, interferon alpha-2a, and interferon alpha-2b.

3. A pharmaceutical composition for treatment of a human individual exposed to or infected with HIV comprising a therapeutically effective amount of mifepristone, one or more therapeutic agents useful for treating HIV infected individuals, selected from the group consisting of zidovudine (AZT), acyclovir, ganciclovir, foscarnet, interferon alpha-2a, and interferon alpha-2b; and a pharmaceutically acceptable carrier therefor.

L4 ANSWER 22 OF 26 USPATFULL on STN

1998:64981 Methods for the identification of compounds capable of inducing the nuclear translocation of a receptor complex comprising the glucocoticoid receptor type II and **viral protein R** interacting protein.

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US 5763190 19980609
APPLICATION: US 1994-309644 19940921 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Human immunodeficiency virus (HIV)-1, HIV-2, and simian immunodeficiency virus contain, in addition to the canonical gag/pol/env genes, additional small open reading frames encoding gene products, including the 96-amino acid 15-kDa virion associated HIV-1 **Vpr** gene product. The conservation of the **vpr** open reading frame in primate lentiviruses suggests that **vpr** is critical to viral replication. A biologically active recombinant HIV-1 **Vpr** protein was employed as a ligand to identify its cellular targets. A novel 41-kDa cytosolic protein was identified and termed the **viral protein R** interacting protein, or Rip-1. Rip-1 displays a wide tissue distribution, including relevant targets of HIV infection. **Vpr** protein induced nuclear translocation of Rip-1, as did glucocorticoid receptor (GR)-II-stimulating steroids. **Vpr** and Rip-1 coimmunoprecipitated with the human GR as part of a receptor complex. The present invention discloses methods for the identification of compounds capable of inducing GR-II/Rip-1 receptor complex cytosolic to nuclear translocation.

CLM What is claimed is:

1. An in vitro method of identifying compounds that induce glucocorticoid receptor type II (GR-II) and **viral protein R** interacting protein (Rip-1) complex translocation comprising the following steps: a) preparing cells expressing Rip-1 and the GR-II wherein Rip-1 and GR-II are capable of forming a cytosolic Rip-1/GR-II receptor complex; b) contacting said cells with a test compound capable of inducing Rip-1/GR-II receptor complex cytoplasmic to nuclear translocation; c) detecting the level of Rip-1/GR-II receptor cytoplasmic to nuclear translocation in said cells in the presence of the test compound; and, d) performing a control assay that detects the level of Rip-I/GR-II receptor complex nuclear translocation in the absence of said test compound; wherein detection of a higher level of cytoplasmic to nuclear translocation of the Rip-1/GR-II receptor complex is indicative of said compound being capable of inducing Rip-1/GR-II receptor complex translocation.

2. The method of claim 1 wherein Rip-1/GR-II receptor complex nuclear translocation is detected through the subcellular fractionation of said cells into soluble and insoluble antigen-containing fractions, followed by the addition of Rip-1-specific **antibodies** to each fraction.

3. The method of claim 2 wherein said **antibodies** contain a radioactive label, fluorescent label, or enzymatic label.

4. The method of claim 2 wherein the soluble and insoluble antigen-containing fractions are attached to a solid support.

5. An in vitro method of inducing glucocorticoid receptor type II (GR-II) and **viral protein R** interacting protein (Rip-1) complex nuclear translocation in cells comprising the following steps: a) preparing cells expressing Rip-1 and GR-II wherein said proteins are capable of forming a cytosolic Rip-1/GR-II receptor complex; and, b) contacting said cells with the human immunodeficiency virus type 1 (HIV-1) **Vpr** protein under conditions wherein said **Vpr** protein binds to the cytosolic Rip-1/GR-II receptor complex thereby generating a **Vpr**/Rip-1/GR-II complex that subsequently undergoes cytoplasmic to nuclear translocation.

L4 ANSWER 23 OF 26 USPATFULL on STN
1998:39510 Compositions and methods for delivery of genetic material.
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Wang, Bin, Beijing, China
**Weiner, David B.**, Merion, PA, United States
Apollon, Inc., Malvern, PA, United States (U.S. corporation)The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5739118 19980414
APPLICATION: US 1994-221579 19940401 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods of introducing genetic material into cells of an individual and compositions and kits for practicing the same are disclosed. The methods comprise the steps of contacting cells of an individual with a genetic vaccine facilitator and administering to the cells, a nucleic acid molecule that is free of retroviral particles. The nucleic acid molecule comprises a nucleotide sequence that encodes a protein that comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen or an antigen associated with a hyperproliferative or autoimmune disease, a protein otherwise missing from the individual due to a missing, non-functional or partially functioning gene, or a protein that produce a therapeutic effect on an individual. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

What is claimed is:

1. A pharmaceutical composition comprising: I) DNA molecules dissociated from infectious agents, said DNA molecules comprising a nucleotide sequence which encodes a protein selected from the group consisting of: a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen; a protein which comprises an epitope identical or substantially similar to an epitope of a protein associated with hyperproliferating cells; and a protein which comprises an epitope identical or substantially similar to an epitope of a protein associated with cells that characterize an autoimmune disease; and ii) a saponin.

2. A pharmaceutical kit comprising: i) a first container that comprises DNA molecules dissociated from infectious agents, said DNA molecules comprising a nucleotide sequence which encodes a protein selected from the group consisting of: a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen; a protein which comprises an epitope identical or substantially similar to an epitope of a protein associated with hyperproliferating cells; a protein which comprises an epitope identical or substantially similar to an epitope of a protein associated with cells that characterize an autoimmune disease; and ii) a second container that comprises a saponin.

3. A method of immunizing an individual comprising the steps of: injecting into tissue of said individual at a site on said individual's body, saponin and a DNA molecule that comprises a DNA sequence that encodes a protein, wherein said DNA sequence is operatively linked to regulatory sequences which control the expression of said DNA sequence in cells of said individual; and wherein said DNA molecule is dissociated from an infectious agent and is taken up by cells in said tissue, said DNA sequence is expressed in said cells and an immune response is generated against said protein.

4. The method of claim 3 wherein said saponin and said DNA molecule are injected into skeletal muscle tissue at a site on said individual's body.

5. The method of claim 3 wherein said protein comprises an epitope of a pathogen antigen.

6. The method of claim 5 wherein said pathogen is a virus.

7. The method of claim 3 wherein said protein is a pathogen antigen.

8. The method of claim 3 wherein said protein comprises an epitope of a hyperproliferative disease-associated protein.

9. The method of claim 3 wherein said protein is a hyperproliferative disease-associated protein.

10. The method of claim 3 wherein said protein comprises an epitope of an autoimmune disease-associated protein.

11. The method of claim 3 wherein said protein is an autoimmune disease-associated protein.

12. The method of claim 3 wherein between 0.01 and 100 mg saponin are administered.

13. The method of claim 3 wherein between 0.1 and 10 mg saponin are administered.

14. The method of claim 3 wherein between 1 ng and 1 mg DNA molecules are administered.

15. The method of claim 3 wherein between 1 μg and 350 μg DNA molecules are administered.

16. The pharmaceutical composition of claim 1 wherein the protein is selected from the group consisting of: a pathogen antigen; a hyperproliferating disease-associated protein; and an autoimmune disease-associated protein.

17. The pharmaceutical composition of claim 1 wherein the protein is a pathogen antigen.

18. The pharmaceutical composition of claim 1 comprising between 0.1 and 10 mg saponin and 1 μg and 350 μg DNA.

19. The pharmaceutical composition of claim 1 comprising between 0.01 and 100 mg saponin and 1 ng and 1 mg DNA.

20. The pharmaceutical kit of claim 2 wherein the protein is selected from the group consisting of: a pathogen antigen; a hyperproliferating disease-associated protein; and an autoimmune disease-associated protein.

21. The pharmaceutical kit of claim 2 wherein the protein is a pathogen antigen.

22. The pharmaceutical kit of claim 2 wherein said first container comprises between 0.01 and 100 mg saponin and said second container comprises 1 ng and 1 mg DNA.

23. The pharmaceutical kit of claim 2 wherein said first container comprises between 0.1 and 10 mg saponin and said second container comprises 1 μg and 350 μg DNA.

L4 ANSWER 24 OF 26 USPATFULL on STN
1998:11867 Chimeric envelope proteins for viral targeting.
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Williams, William, Havertown, PA, United States
Levy, David N., Philadelphia, PA, United States
The Wistar Institute of Anatomy & Biology, Philadelphia, PA, United States (U.S. corporation)The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5714316 19980203
APPLICATION: US 1993-147890 19931104 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides compositions and methods for targeting recombinant retroviral particles specifically to cells of interest for delivery of desired therapeutic or toxic agents. The invention provides chimeric nucleotide constructs, chimeric proteins formed of a selected viral envelope gene from which a selected sequence has been deleted and into which has been inserted all or an effective portion of a heterologous ligand, said ligand or portion thereof capable of binding to a selected receptor, recombinant viral particles formed of the chimeric proteins, a biological mediator for delivery to the target cell; and retroviral gag and pol proteins. The lack of retroviral nucleic acid renders the viral particle replication defective and non-pathogenic.
CLM What is claimed is:
1. A chimeric nucleotide construct comprising an HIV gp160 envelope gene from which the sequence encoding about amino acid 392 to about amino acid 446 or a fragment thereof corresponding to the HIV binding site to CD4 has been deleted and into which has been inserted a heterologous sequence encoding a ligand, said ligand selected from the group consisting of GMCSF and CD4.

2. The construct according to claim 1 wherein said HIV is selected from the group consisting of HIV-1 and HIV-2.

3. The construct according to claim 2 wherein said HIV-2 is the isolate HIV-2 ROD.

4. A chimeric nucleotide construct SEQ ID NO:1.

5. A chimeric nucleotide construct SEQ ID NO:2.

6. A vector comprising: a chimeric nucleotide construct comprising an HIV gp160 envelope gene from which the sequence encoding about amino acid 392 to about amino acid 446 or a fragment thereof corresponding to the HIV binding site to CD4 has been deleted and into which has been inserted a heterologous sequence encoding a ligand selected from the group consisting of GMCSF and CD4; and a regulatory sequence which directs expression of the chimeric construct.

7. A host cell transformed with the vector of claim 6.

8. A method for producing a chimeric protein comprising culturing a host cell according to claim 7 and recovering the expressed chimeric protein.

9. A recombinant retroviral particle comprising: (a) a chimeric protein comprising an HIV gp160 envelope from which about amino acid 392 to about amino acid 446 or a fragment thereof corresponding to the HIV binding site to CD4 has been deleted and into which has been inserted a heterologous sequence encoding a ligand selected from the group consisting of GMCSF and CD4; (b) a biological mediator gene sequence for delivery to the target cell; (c) a retroviral gag protein; (d) a retroviral pol protein; wherein the particle lacks HIV nucleic acid, rendering it non-pathogenic and incapable of recombination.

10. The retroviral particle according to claim 9 wherein the mediator is selected from the group consisting of a gene product, a diagnostic label, and a toxic agent.

11. A host cell transformed with the vector of claim 6, wherein said cell further comprises: (a) a gene encoding a biological mediator selected from the group consisting of a gene product and a toxic agent; (b) a retrovital packaging sequence; and (c) a retrovital gag gene and a retrovital pol gene in operative association with regulatory sequences capable of directing the replication and expression thereof.

L4 ANSWER 25 OF 26 USPATFULL on STN

97:51852 Method and kit for identification of antiviral agents capable of abrogating HIV **Vpr**-Rip-1 binding interactions.

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The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)

US 5639598 19970617
APPLICATION: US 1994-246177 19940519 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Human and simian immunodeficiency viruses (HIV/SIVs) contain, in addition to the canonical gag/pol/env genes, additional small open reading frames (ORFs) encoding gene products, including the 96-amino acid 15-kDa virion-associated HIV-1 **Vpr** gene product. **Vpr** functions as a regulator of cellular processes related to HIV replication. A biologically active recombinant HIV-1 **Vpr** protein was employed as a ligand to identify its cognate cellular target(s). A novel 41-kDa cytosolic **viral protein R** interacting protein, designated Rip-1, was identified using the recited assay. Rip-1 displays a wide-tissue

distribution, including relevant targets of HIV infection. HIV-1 **Vpr** induced nuclear translocation of Rip-1. This invention provides novel biochemical reagents and methods that will facilitate the identification of antiviral agents.

CLM What is claimed is:

1. An in vitro method of identifying a compound which is capable of preventing HIV-1 **Vpr** from forming a complex with Rip-1 comprising the following steps: a) contacting **Vpr** and Rip-1 in the presence of said compound; b) determining the level of **Vpr**/Rip-1 complex formation; c) comparing the level of **Vpr**/Rip-1 complex formation in step (b) to the level of **Vpr**/Rip-1 binding that occurs in the absence of said test compound.

2. The method of claim 1 wherein said level of **Vpr**/Rip-1 complex formation is determined by immunoassay.

3. An in vitro method of identifying a compound which is capable of preventing HIV-1 **Vpr** from forming a complex with Rip-1 comprising the following steps: a) immobilizing Rip-1 to a solid support; b) contacting said immobilized Rip-1 with **Vpr** and a test compound; c) measuring **Vpr** binding to immobilized Rip-1 through the administration of **Vpr**-specific **antibodies**; and, d) comparing the level of **Vpr**/Rip-1 binding in step (c) to the level of **Vpr**/Rip-1 binding that occurs in the absence of said test compound.

4. An in vitro method of identifying a compound which is capable of preventing HIV-1 **Vpr** from forming a complex with Rip-1 comprising the following steps: a) immobilizing **Vpr** to a solid support; b) contacting said immobilized **Vpr** with Rip-1 and a test compound; c) measuring Rip-1 binding to immobilized **Vpr** through the administration of Rip-1-specific **antibodies**; and, d) comparing the level of **Vpr**/Rip-1 binding in step (c) to the level of **Vpr**/Rip-1 binding that occurs in the absence of said test compound.

5. An in vitro method for the identification of compounds capable of inhibiting HIV-1 **Vpr** from forming a complex with Rip-1, said method comprising the following steps: a) contacting a culture of HIV-1-infected cells with a test compound; b) determining the level of **Vpr**/Rip-1 binding in said HIV-1infected cells; and, c) comparing the level of **Vpr**/Rip-1 binding in step (b) to the level of **Vpr**/Rip-1 binding that occurs in HIV-1-infected cells cultured in the absence of said test compound.

6. An in vitro method for the identification of compounds capable of inhibiting HIV-1 viral replication through the abrogation of HIV-1Vpr/Rip-1 complex cytoplasmic to nuclear translocation, said method comprising the following steps: a) contacting a culture of HIV-1-infected cells with a test compound; b) determining the level of cytoplasmic to nuclear translocation of HIV-1 **Vpr**/Rip-1 complexes; and, c) comparing the level of cytoplasmic to nuclear translocation of HIV-1 **Vpr**/Rip-1 complexes in the presence of said test compound to the level of cytoplasmic to nuclear translocation of HIV-1 **Vpr**/Rip-1 complexes that occurs in HIV-1-infected cells cultured in the absence of said test compound.

7. A method according to claim 6 further comprising the following steps: d) determining the level of p24 antigen produced in HIV-1 infected cells cultured in the presence of said test compound; e) comparing the level of p24 antigen produced in HIV-1-infected cells cultured in the presence of said test compound to the level of p24 antigen produced by HIV-1-infected cells cultured in the absence of said test compound; wherein said comparison results in the identification of compounds capable of inhibiting HIV-1 viral replication.

8. An in vitro method for the identification of glucocorticoid receptor antagonists capable of inhibiting HIV-1 **Vpr** from forming a complex

culture of HIV-1-infected cells with a glucocorticoid receptor antagonist; b) determining the level of **Vpr**/Rip-1 binding in said HIV-1 infected cells; and, c) comparing the level of **Vpr**/Rip-1 binding in step (b) to the level of **Vpr**/Rip-1 binding that occurs in HIV-1-infected cells cultured in the absence of said test compound.

9. An in vitro method for the identification of glucocorticoid receptor antagonists capable of inhibiting HIV-1 viral replication through the abrogation of HIV-1 **Vpr**/Rip-1 complex cytoplasmic to nuclear translocation, said method comprising the following steps: a) contacting a culture of HIV-1-infected cells with a glucocorticoid receptor antagonist; b) determining the level of cytoplasmic to nuclear translocation of HIV-1 **Vpr**/Rip-1 complexes; and, c) comparing the level of cytoplasmic to nuclear translocation of HIV-1 **Vpr**/Rip-1 complexes in the presence of said test compound to the level of cytoplasmic to nuclear translocation of HIV-1 **Vpr**/Rip1 complexes that occurs in HIV-1-infected cells cultured in the absence of said test compound.

L4 ANSWER 26 OF 26 USPATFULL on STN
97:3820 Genetic immunization.
**Weiner, David B.**, Merion, PA, United States
Williams, William V., Havertown, PA, United States
Wang, Bin, Havertown, PA, United States
The Wistar Institute, Philadelphia, PA, United States (U.S. corporation)The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 5593972 19970114
APPLICATION: US 1993-125012 19930921 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods of prophylactic and therapeutic immunization of an individual against pathogen infection, diseases associated with hyperproliferative cells and autoimmune diseases are disclosed. The methods comprise the steps of administering to cells of an individual, a nucleic acid molecule that comprises a nucleotide sequence that encodes a protein which comprises at least one epitope that is identical or substantially similar to an epitope of a pathogen antigen, a hyperproliferative cell associated protein or a protein associated with autoimmune disease respectively. In each case, nucleotide sequence is operably linked to regulatory sequences to enable expression in the cells. The nucleic acid molecule is free of viral particles and capable of being expressed in said cells. The cells may be contacted cells with a cell stimulating agent. Methods of prophylactically and therapeutically immunizing an individual against HIV are disclosed. Pharmaceutical compositions and kits for practicing methods of the present invention are disclosed.

CLM What is claimed is:
1. A method of immunizing an individual comprising: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes an antigen from a pathogen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

2. The method of claim 1 wherein said pathogen is an intracellular pathogen.

3. The method of claim 1 wherein said pathogen is a virus selected from the group consisting of: human immunodeficiency virus, HIV; human T cell leukemia virus, HTLV; influenza virus; hepatitis A virus; hepatitis B virus; hepatitis C virus; human papilloma virus, HPV; Herpes simplex 1 virus, HSV1; Herpes simplex 2 virus, HSV2; Cytomegalovirus, CMV;

Epstein Barr virus, EBV, rhinovirus, and coronavirus.

4. The method of claim 1 wherein said pathogen is HIV and said DNA molecule comprises a DNA sequence that encodes an HIV antigen.

5. The method of claim 1 wherein at least two non-identical DNA molecules are injected into skeletal muscle tissue of said individual at different sites on said individual's body, said bupivacaine being injected into each of the different sites of an individual; said non-identical DNA molecules each comprising DNA sequences encoding one or more pathogen antigens of the same pathogen.

6. A method of immunizing an individual comprising: injecting into skeletal muscle tissue of said individual at a site on said individual's body, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes a hyperproliferative disease-associated protein operatively linked to regulatory sequences; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells, and an immune response is generated against said hyperproliferative disease-associated protein.

7. The method of claim 6 wherein said DNA molecule comprises a DNA sequence encoding a target protein selected from the group consisting of: protein products of oncogenes myb, myc, fyn, ras, src, neu and trk; protein products of translocation gene bcr/abl; P53; variable regions of **antibodies** made by B cell lymphomas; and variable regions of T cell receptors of T cell lymphomas.

8. A method of immunizing an individual comprising: injecting into skeletal muscle tissue of said individual, bupivacaine and a DNA molecule that comprises a DNA sequence that encodes an autoimmune disease-associated protein operatively linked to regulatory sequences; wherein said DNA molecule is taken up by cells in said skeletal muscle tissue, said DNA sequence is expressed in said cells and an immune response is generated against said autoimmune disease-associated protein.

9. The method of claim 8 wherein said DNA molecule comprises a DNA sequence encoding a target protein selected from the group consisting of: variable regions of **antibodies** involved in B cell mediated autoimmune disease; and variable regions of T cell receptors involved in T cell mediated autoimmune disease.

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=> d his

     (FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
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L1            40 S E5
L2            49 S E3 OR E5
L3            26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4            26 S L3 AND ANTIBOD?

=> e levy david/in
E1             1     LEVY DANIELE/IN
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E3            23 --> LEVY DAVID/IN
E4             4     LEVY DAVID E/IN
E5             5     LEVY DAVID F/IN
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E7            63     LEVY DAVID H/IN
E8             1     LEVY DAVID HENRY/IN
E9             1     LEVY DAVID HOWARD/IN
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E12           1      LEVY DAVID M E/IN

=> s e3
L5  .        23 "LEVY DAVID"/IN

=> s l5 not l4
L6           23 L5 NOT L4

=> d l6,ti,1-23

L6   ANSWER 1 OF 23  USPATFULL on STN
TI     Method and system for dynamically personalizing transportation in a
       vehicle

L6   ANSWER 2 OF 23  USPATFULL on STN
TI     Immortalized, homozygous stat1-deficient mammalian cell lines and their
       uses

L6   ANSWER 3 OF 23  USPATFULL on STN
TI     Method of customizing a browsing experience on a world-wide-web site

L6   ANSWER 4 OF 23  USPATFULL on STN
TI     Method of customizing a user's browsing experience on a World-Wide-Web
       site

L6   ANSWER 5 OF 23  USPATFULL on STN
TI     Hybrid networking system

L6   ANSWER 6 OF 23  USPATFULL on STN
TI     System and method for improving polarization matching on a cellular
       communication forward link

L6   ANSWER 7 OF 23  USPATFULL on STN
TI     Cellular base station augmentation system and method

L6   ANSWER 8 OF 23  USPATFULL on STN
TI     System and method for providing polarization matching on a cellular
       communication forward link

L6   ANSWER 9 OF 23  USPATFULL on STN
TI     Medical simulation apparatus and related method

L6   ANSWER 10 OF 23  USPATFULL on STN
TI     Privacy and security method and system for a World-Wide-Web site

L6   ANSWER 11 OF 23  USPATFULL on STN
TI     Compact keyed input device

L6   ANSWER 12 OF 23  USPATFULL on STN
TI     Sport target device and method

L6   ANSWER 13 OF 23  USPATFULL on STN
TI     Products having anti-microbial activity

L6   ANSWER 14 OF 23  USPATFULL on STN
TI     Device and method for securing shafts adjustably within tubes,
       especially bicycle components

L6   ANSWER 15 OF 23  USPATFULL on STN
TI     Clip-on tape dispenser for reeled tape

L6   ANSWER 16 OF 23  USPATFULL on STN
TI     Compact keypad system and method
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TI     Cleaning assembly

L6   ANSWER 18 OF 23  USPATFULL on STN
TI     Optically transmissive armature for cursor control device

L6   ANSWER 19 OF 23  USPATFULL on STN
TI     Holder for flexible sheet material

L6   ANSWER 20 OF 23  USPATFULL on STN
TI     Gaming apparatus

L6   ANSWER 21 OF 23  USPATFULL on STN
TI     Roulette gaming apparatus

L6   ANSWER 22 OF 23  USPATFULL on STN
TI     Data-processing system for determining gains and losses from bets

L6   ANSWER 23 OF 23  USPATFULL on STN
TI     DATA-PROCESSING SYSTEM FOR DETERMINING GAINS AND LOSSES FROM BETS


=> e refaeli yosef/in
E1            9     REFAELI MOSHE/IN
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E3            6 --> REFAELI YOSEF/IN
E4            2     REFAELY ISAAC/IN
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E8            2     REFAI WAIL M/IN
E9            2     REFAIT DENIS/IN
E10           1     REFALO ANTHONY/IN
E11           1     REFALO III JOHN/IN
E12           2     REFALO III JOHN J/IN

=> s e3
L7            6 "REFAELI YOSEF"/IN

=> d his

     (FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
                E WEINER DAVID/IN
L1            40 S E5
L2            49 S E3 OR E5
L3            26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4            26 S L3 AND ANTIBOD?
                E LEVY DAVID/IN
L5            23 S E3
L6            23 S L5 NOT L4
                E REFAELI YOSEF/IN
L7             6 S E3

=> s l7 not (l5 or l1)
L8            0 L7 NOT (L5 OR L1)

=> e montagnier luc/in
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E3           99 --> MONTAGNIER LUC/IN
E4           12     MONTAGNINO JAMES/IN
E5           66     MONTAGNINO JAMES G/IN
E6            1     MONTAGNINO JIM/IN
E7            1     MONTAGNINO LUCIAN/IN
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E10           2      MONTAGNON BRUNO/IN
E11           1      MONTAGNON FRANCOISE/IN
E12           3      MONTAGNON JACQUES/IN

=> s e3
L9           99 "MONTAGNIER LUC"/IN

=> s l9 and (Vpr or viral protein R)
          1091 VPR
         65975 VIRAL
        174530 PROTEIN
        977413 R
            32 VIRAL PROTEIN R
                 (VIRAL(W)PROTEIN(W)R)
L10          9 L9 AND (VPR OR VIRAL PROTEIN R)

=> d l10,cbib,ab,clm,1-9

L10  ANSWER 1 OF 9  USPATFULL on STN
2002:188123 Variant of LAV viruses.
    Alizon, Marc, Paris, FRANCE
    Sonigo, Pierre, Paris, FRANCE
    Wain-Hobson, Simon, Montigny les Bretonneux, FRANCE
    Montagnier, Luc, Le Plessis Robinson, FRANCE
    Institut Pasteur, FRANCE (non-U.S. corporation)
    US 6426073 B1 20020730
    APPLICATION: US 1999-328438  19990609 (9)
    PRIORITY: FR 1986-401380  19860623
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The present invention relates to a novel human immunodeficiency virus
       (HIV) capable of inducing lymphadenopathies (LAS) and acquired immune
       deficiency syndromes (AIDS) in patients which has been designated the
       lymphadenopathy associated virus strain MAL ($LAV_{MAL}$). Although the
       overall genomic organization of $LAV_{MAL}$ is similar to other known
       HIV-1 isolates such as $LAV_{BRU}$ and HTLV-III, nevertheless, this
       virus also displays considerable genotypic and phenotypic diversity as
       compared to these isolates. A proviral molecular clone of the virus was
       obtained and characterized. The complete nucleotide sequence of this
       clone was ascertained and putative regulatory regions (e.g., U3, R,
       U5,), regulatory elements (e.g., the TATA box, AATAAA polyadenylation
       signal, primer binding site), and open reading frames (e.g., Gag, Pol,
       Env, Vif, Vpr, Tat, Rev, Nef) identified. Of particular interest are
       unique polypeptides derived from the viral envelope. The claimed
       invention is directed toward isolated $LAV_{MAL}$ Env polypeptides
       consisting of 5-150 amino acids wherein said peptides contain a
       $LAV_{MAL-specific}$ epitope. These peptides will prove useful, inter
       alia, as diagnostic reagents and in the generation of immunological
       reagents for the detection of the virus.
CLM    What is claimed is:
       1. An isolated HIV-1 $LAV_{MAL}$ Env polypeptide consisting of 5-150
       amino acid residues as set forth in FIGS. 3E-3F, wherein said peptide
       contains a $LAV_{MAL-specific}$ antigenic determinant.

       2. The peptide of claim 1, wherein said peptide is generated by chemical
       cleavage.

       3. The peptide of claim 1, wherein said peptide is expressed from a
       recombinant DNA.

       4. The peptide of claim 1, wherein said peptide is generated by chemical
       synthesis.
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3. The peptide of claim 1, wherein said peptide binds to antibodies in AIDS patient sera; and wherein said antibodies are capable of binding to viral antigens encoded by the $LAV_{MAL}$ molecular clone having C.N.C.M. accession number I-641.

L10 ANSWER 2 OF 9 USPATFULL on STN
2002:51108 CLONED DNA SEQUENCES RELATED TO THE ENTIRE GENOMIC RNA OF HUMAN IMMUNODEFICIENCY VIRUS II (HIV-2), POLYPEPTIDES ENCODED BY THESE DNA SEQUENCES AND USE OF THESE DNA CLONES AND POLYPEPTIDES IN DIAGNOSTIC KITS.
Alizon, Marc, Paris, FRANCE
**Montagnier, Luc**, Le Plessis Robinson, FRANCE
Geutard, Denise, Paris, FRANCE
Clavel, Francois, Rockville, MD, United States
Sonigo, Pierre, Paris, FRANCE
Guyader, Mireille, Paris, FRANCE
Institut Pasteur, Paris, FRANCE (non-U.S. corporation)
US 6355789 B1 20020312
APPLICATION: US 1995-468424 19950606 (8)
PRIORITY: FR 1986-911 19860122
FR 1986-1635 19860206
FR 1986-1985 19860213
FR 1986-3881 19860318
FR 1986-4215 19860324
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed toward nucleic acids containing the full-length human immunodeficiency virus type 2 ROD ($HIV\text{-}2_{ROD}$) pol gene. HIV-2, which was originally designated lymphadenopathy-associated virus type II (LAV-II), was isolated from AIDS patients in West Africa. The virus is genotypically and phenotypically distinct from HIV-1 and bears a closer genetic relationship to the simian immunodeficiency virus (SIV). The present invention describes the preparation of $HIV\text{-}2_{ROD}$ proviral molecular clones from a genomic lambda phage library of CD4+-infected cells. The complete nucleotide sequence of the full-length genome was determined and the putative gag, pol, env, vif (Q), **vpr** (R), vpx (X), nef (F), tat, and rev (art) genes identified. The claimed invention is directed toward nucleic acids containing the full-length $HIV\text{-}2_{ROD}$ pol gene (nt 1829-4936). These nucleic acids should prove useful as diagnostic reagents for the detection of HIV-2 and facilitate expression of the pol gene product.
CLM What is claimed is:
1. A nucleic acid of HIV-2 having the nucleotide sequence of a full length pol gene as set forth in FIG. 6.

L10 ANSWER 3 OF 9 USPATFULL on STN
2001:29295 Nucleotide sequences derived from the genome of retroviruses of the HIV-1, HIV-2, and SIV type, and their uses in particular for the amplification of the genomes of these retroviruses and for the in vitro diagnosis of the diseases due to these viruses.
Moncany, Maurice, Paris, France
**Montagnier, Luc**, Le Plessis-Robinson, France
Institut Pasteur, Paris, France (non-U.S. corporation)Institut National de la Sante et de la Recherche Medicale, Paris, France (non-U.S. corporation)
US 6194142 B1 20010227
APPLICATION: US 1998-92077 19980605 (9)
PRIORITY: FR 1989-7354 19890602
FR 1989-12371 19890920
WO 1990-FR390 19900506
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to polypeptides encoded by a nucleotide sequence from an HIV-1, HIV-2, or SIV viral genome, in which the nucleotide sequence is amplified from the viral genome using a pair of

primers that contain sequences that are conserved between different HIV and SIV strains. The primers are insensitive to variations in the genomes of different HIV and SIV isolates and, therefore, can be used to amplify nucleotide sequences from HIV-1, HIV-2, and SIV strains. The invention also relates to antibodies directed against these polypeptides and methods and kits for diagnosing viral infection.

CLM What is claimed is:

1. A polypeptide fragment of a viral protein encoded by a nucleotide sequence from a viral genome selected from the group consisting of HIV-1, HIV-2, and SIV and expressed by a method comprising: a) amplifying the nucleic acid encoding said polypeptide with at least two primers, wherein said first primer is complementary to a region of nucleotides of the nucleic acid of said genome, said second primer is complementary to a region of nucleotides of the strand of DNA complementary to said nucleic acid of said genome, wherein said regions of nucleotides are separated by about 100 to about 1100 base pairs when said complementary strands are hybridized to form one double-stranded nucleic acid, and said primers are selected from the group of nucleotides oriented in the 5' to 3' direction consisting of: nucleotides 636-653, 854-872, 1369-1388, and 2021-2039 of the gag gene of HIV-1 Bru; nucleotides 900-881, 1385-1369, 1388-1369, and 2039-2021 of a nucleic acid sequence complementary to the gag gene of HIV-1 Bru; nucleotides 635-652, 864-888, 1403-1421, and 2055-2073 of the gag gene of HIV-1 Mal; nucleotides 916-897, 1419-1403, 1421-1403, and 2073-2055 of a nucleic acid sequence complementary to the gag gene of HIV-1 Mal; nucleotides 636-653, 848-872, 1369-1388, and 2024-2042 of the gag gene of HIV-1 Eli; nucleotides 900-881, 1385-1369, 1388-1369, and 2042-2024 of a nucleic acid sequence complementary the gag gene of HIV-1 Eli; nucleotides 859-876, 1160-1184, 1687-1706, and 2329-2349 of the gag gene of HIV-2 ROD; nucleotides 1212-1193, 1703-1687, 1706-1687, and 2349-2329 of a nucleic acid sequence complementary to the gag gene of HIV-2 ROD; nucleotides 834-851, 1124-1148, 1651-1670, and 2299-2318 of the gag gene of SIV-MAC; nucleotides 1176-1157, 1667-1651, 1670-1651, and 2381-2299 of a nucleic acid sequence complementary to the gag gene of SIV-MAC; nucleotides 5590-5610 of the **vpr** gene of HIV-1 Bru; nucleotides 5870-5849 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Bru; nucleotides 5585-5605 of the **vpr** gene of HIV-1 Mal; nucleotides 5865-5844 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Mal; nucleotides 5554-5574 of the **vpr** gene of HIV-1 Eli; nucleotides 5834-5813 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Eli; nucleotides 6233-6296 of the **vpr** gene of HIV-2 ROD; nucleotides 6551-6531 of a nucleic acid sequence complementary to the **vpr** gene of HIV-2 ROD; nucleotides 6147-6170 of the **vpr** gene of SIV-MAC; nucleotides 6454-6431 of a nucleic acid sequence complementary to the **vpr** gene of SIV-MAC; nucleotides 2620-2643, 3339-3361, 4186-4207, and 4992-5011 of the pol gene of HIV-1 Bru; nucleotides 2643-2620, 3361-3339, 4207-4186, and 5011-4992 of a nucleic acid sequence complementary to the pol gene of HIV-1 Bru; nucleotides 2615-2638, 3333-3356, 4181-4202, and 4987-5006 of the pol gene of HIV-1 Mal; nucleotides 2638-2615, 3356-3334, 4202-4181, and 5006-4987 of a nucleic acid sequence complementary to the pol gene of HIV-1 Mal; nucleotides 2584-2607, 3303-3325, 4150-4171, and 4956-4975 of the pol gene of HIV-1 Eli; nucleotides 2607-2584, 3325-3303, 4171-4150, and 4975-4956 of a nucleic acid sequence complementary to the pol gene of HIV-1 Eli; nucleotides 2971-2994, 3690-3712, 4534-4555, and 5340-5359 of the pol gene of HIV-2 ROD; nucleotides 2994-2971, 3712-3690, 4555-4534, and 5359-5340 of a nucleic acid sequence complementary to the pol gene of HIV-2 ROD; nucleotides 2887-3010, 3606-3628, 4450-4471, and 5256-5275 of the pol gene of SIV-MAC; nucleotides 3010-2887, 3628-3606, 4471-4450, and 5275-5256 of a nucleic acid sequence complementary to the pol gene of SIV-MAC; nucleotides 9165-9185 and 9542-9564 of the nef2 gene of HIV-2 ROD; nucleotides 9564-9542 and 9956-9933 of a nucleic acid sequence complementary to the nef2 gene of HIV-2 ROD; nucleotides 9139-9159 and 9516-9538 of the nef2 gene of SIV-MAC; nucleotides 9538-9516 and 9893-9870 of a nucleic acid sequence complementary to the nef2 gene of SIV-MAC; nucleotides

5775-5754 and 6082-6061 of a nucleic acid sequence complementary to the vif2 gene of HIV-2 ROD; nucleotides 5340-5366 and 5670-5691 of the vif2 gene of SIV-MAC; nucleotides 5691-5670 and 5995-5974 of a nucleic acid sequence complementary to the vif2 gene of SIV-MAC; nucleotides 5900-5918 of the vpx gene of HIV-2 ROD; nucleotides 6228-6208 of a nucleic acid sequence complementary to the vpx gene of HIV-2 ROD; nucleotides 5813-5831 of the vpx gene of SIV-MAC; nucleotides 6141-6121 of a nucleic acid sequence complementary to the vpx gene of SIV-MAC; nucleotides 9116-9136 of the nef1 gene of HIV-1 Bru; nucleotides 9136-9116 and 9503-9483 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Bru; nucleotides 9117-9137 of the nef1 gene of HIV-1 Mal; nucleotides 9137-9117 and 9505-9484 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Mal; nucleotides 9062-9082 of the nef1 gene of HIV-1 Eli; nucleotides 9082-9062 and 9449-9428 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Eli; nucleotides 5073-5099 and 5383-5405 of the vif1 gene of HIV-1 Bru; nucleotides 5405-5383 and 5675-5653 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Bru; nucleotides 5068-5094 and 5378-5400 of the vif1 gene of HIV-1 Mal; nucleotides 5400-5378 and 5670-5648 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Mal; nucleotides 5037-5063 and 5347-5369 of the vif1 gene of HIV-1 Eli; nucleotides 5369-5347 and 5639-5617 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Eli; nucleotides 6081-6105 and 6240-6263 of the vpu gene of HIV-1 Bru; nucleotides 6343-6321 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Bru; nucleotides 6076-6100 and 6238-6261 of the vpu gene of HIV-1 Mal; nucleotides 6338-6316 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Mal; nucleotides 6045-6069 and 6207-6230 of the vpu gene of HIV-1 Eli; and nucleotides 6307-6285 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Eli; b) introducing said amplified nucleotide sequence into a vector; c) transforming a host cell with said vector; and d) placing said transformed host cell in culture and recovering said polypeptide fragment from said culture.

2. A polypeptide fragment of a viral protein encoded by a nucleotide sequence from a viral genome selected from the group consisting of HIV-1, HIV-2, and SIV and expressed by a method comprising: a) amplifying the nucleic acid encoding said polypeptide with at least two primers, wherein said first primer is complementary to a region of nucleotides of the nucleic acid of said genome, said second primer is complementary to a region of nucleotides of the strand of DNA complementary to said nucleic acid of said genome, wherein said regions of nucleotides are separated by about 100 to about 1100 base pairs when said complementary strands are hybridized to form one double-stranded nucleic acid, and said primers are selected from the group consisting of: TBL MMy1:TGG CGC CCG AAC AGG GAC (SEQ ID NO:1); TGG CGC CTG AAC AGG GAC (SEQ ID NO:2);MMy2:GGC CAG GGG GAA AGA AAA A (SEQ ID NO:3); GGC CCG GCG GAA AGA AAA A (SEQ ID NO:4); GGC CCG GAG GAA AGA AAA A (SEQ ID NO:5);MMy3:TGC CCA TAC AAA ATG TTT TA (SEQ ID NO:6); TGC CCA CAC TAT ATG TTT TA (SEQ ID NO:7);MMy4:TGC ATG GCT GCT TGA TG (SEQ ID NO:8); TGC ATA GCT GCC TGG TG (SEQ ID NO:9);MMy4B:CTT TCG ATG GCT GCT TGA TG (SEQ ID NO:10); CTC TGC ATA GCT GCT TGC TG (SEQ ID NO:11);MMy4Ba:CAT CAA GCA GCC ATG CAA AG (SEQ ID NO:12); CAC CAG GCA GCT ATG CAG AG (SEQ ID NO:13);MMy28:AGG GCT GTT GGA AAT GTG G (SEQ ID NO:14); AGG GCT GTT GGA AGT GTG G (SEQ ID NO:15);MMy28a:CCA CAT TTC CAG CAT CCC T (SEQ ID NO:16); CCA CAT TTC CAG CAG CCC T (SEQ ID NO:17); CCA CAT TTC CAG CAC CCC T (SEQ ID NO:18);MMy18:GAT AGA TGG AAC AAG CCC CAG (SEQ ID NO:19);MMy19:TCC ATT TCT TGC TCT CCT CTG T (SEQ ID NO:20);MMy29:TAA AGC CAG GAA TGG ATG GCC CAA (SEQ ID NO:21); TAA AGC CAG GAA TGG ATG GAC CAA (SEQ ID NO:22);MMy29a:TTG GGC CAT CCA TTC CTG GCT TTA (SEQ ID NO:23);

ATA CAG AA (SEQ ID NO:25); TGG
ACT GTC AAT GAT ATA CAG AA (SEQ ID NO:26);MMy30a:TTC TGT ATG TCA TTG ACA
GTC CA (SEQ ID NO:27); TTC TGT
ATG TCA TTG ACT GTC CA (SEQ ID NO:28);MMy31:CAT GGG TAC CAG CAC ACA AAG
G (SEQ ID NO:29);MMy31a:CCT TTG TGT GCT GGT ACC CAT G (SEQ ID
NO:30);MMy32:TGG AAA GGT GAA GGG GCA GT (SEQ ID NO:31);
TGG AAA GGT GAA GGA GCA GT (SEQ ID NO:32);MMy32a:ACT GCC CCT TCA CCT TTC
CA (SEQ ID NO:33); ACT GCC CCT
TCT CCT TTC CA (SEQ ID NO:34);
ACT GCC CCT TCC CCT TTC CA (SEQ ID NO:35);MMy12:AGA GAC TCT TGC GGG CGC
GTG (SEQ ID NO:36);MMy13:ATA TAC TTA GAA AAG GAA GAA GG (SEQ ID
NO:37);MMy13a:CCT TCT TCC TTT TCT AAG TAT AT (SEQ ID NO:38);MMy14:AGC
TGA GAC AGC AGG GAC TTT CCA (SEQ ID NO:39);MMy20:TAT GGA GGA GGA AAA GAG
ATG GAT AGT (SEQ ID NO:40);MMy21:TAG CAC TTA TTT CCC TTG CTT T (SEQ ID
NO:41);MMy21a:AAA GCA AGG GAA ATA AGT GCT A (SEQ ID NO:42);MMY22:CCC TTG
TTC ATC ATG CCA GTA T (SEQ ID NO:43);MMy23:ATG TCA GAT CCC AGG GAG A
(SEQ ID NO:44);MMy24:CCT GGA GGG GGA GGA GGA GGA (SEQ ID
NO:45);MMy10:AAA AGA AAA GGG GGG ACT GGA (SEQ ID NO:58);MMy10a:TCC AGT
CCC CCC TTT TCT TTT (SEQ ID NO:59);MMy11:AAA GTC CCC AGC GGA AAG TCC C
(SEQ ID NO:60);MMy15:GAT TAT GGA AAA CAG ATG GCA GGT GAT (SEQ ID
NO:61);MMy16:GCA GAC CAA CTA ATT CAT CTG TA (SEQ ID NO:62);MMy16a:TAC
AGA TGA ATT AGT TGG TCT GC (SEQ ID NO:63);MMy17:CTT AAG CTC CTC TAA AAG
CTC TA (SEQ ID NO:64);MMy25:GTA AGT AGT ACA TGT AAT GCA ACC T (SEQ ID
NO:65);MMy26:AGC AGA AGA CAG TGG CCA TGA GAG (SEQ ID NO:66);andMMy27:ACT
ACA GAT CAT CAA TAT CCC AA (SEQ ID NO:67); b) introducing said amplified nucleotide sequence into a vector; c) transforming a host cell with said vector; and d) placing said transformed host cell in culture and recovering said polypeptide fragment from said culture.

3. An antibody capable of binding to the polypeptide of claim 1 or 2.

4. A method for the in vitro diagnosis of the infection of a mammal by a virus of the HIV-1, HIV-2, or SIV type, said virus comprising at least one polypeptide antigen, said method comprising placing a biological sample taken from said mammal in contact with the antibody according to claim 3, and detecting the immunological complex formed between said antigen and said antibody.

5. A kit for the diagnosis of infection of a mammal by a virus of the HIV-1, HIV-2, or SIV type, said kit comprising an antibody according to claim 3 and reagents for the detection of the immunological complex formed between said antibody and said antigen.

6. A composition comprising at least one polypeptide according to claim 1 in combination with a pharmaceutically acceptable vehicle.

7. A composition comprising at least one polypeptide according to claim 2 in combination with a pharmaceutically acceptable vehicle.

L10 ANSWER 4 OF 9 USPATFULL on STN
2000:170665 Human immunodeficiency virus type 2 (HIV-2) polypeptides and methods of producing them.
Alizon, Marc, Paris, France
**Montagnier, Luc**, Le Plessis Robinson, France
Guetard, Denise, Paris, France
Clavel, Francois, Rockville, MD, United States
Sonigo, Pierre, Paris, France
Guyader, Mireille, Paris, France
Institut Pasteur, Paris, France (non-U.S. corporation)
US 6162439 20001219
APPLICATION: US 1995-466707 19950606 (8)
PRIORITY: FR 1986-911 19860122
FR 1986-1635 19860206
FR 1986-1985 19860213

FR 1986-4215 19860324
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention is directed toward polypeptides derived from novel lentiviruses. A novel lentivirus, designated the human immunodeficiency virus type 2, was isolated from West African patients with acquired immune deficiency syndrome (AIDS). Several isolates were obtained and designated HIV-$2_{ROD}$, HIV-$2_{IRMO}$, and HIV-$2_{EHO}$. A recombinant lambda phage library was constructed from HIV-$2_{ROD}$ -infected CEM genomic DNA. Overlapping molecular clones were obtained and the nucleotide sequence of the complete 9.5-kilobase (kb) HIV-$2_{ROD}$ genome ascertained. The genetic organization of HIV-2 is analogous to that of other retroviruses and consists of the 5'LTR-gag-pol-central region-env-nef-3'LTR. The central region also encodes for the regulatory proteins Tat and Rev, as well as the ancillary proteins Vif, **Vpr**, and Vpx. The proteins encoded by this proviral clone will provide novel immunologic, biochemic, and diagnostic reagents useful for the detection of HIV-2.
CLM What is claimed is:
1. A purified polypeptide of HIV-2 selected from the group consisting of p16, p26, p12, Pol, Vif, **Vpr**, Vpx, Env, Tat, Rev, and Nef.

2. An immunizing agent comprising at least one polypeptide of HIV-2 selected from the group consisting of p16, p26, p12, Pol, Vif, **Vpr**, Vpx, Env, Tat, Rev, and Nef; and a pharmaceutically acceptable vehicle.

3. A method for producing a polypeptide as claimed in claim 1, comprising, obtaining a DNA coding for the amino acid sequence of the polypeptide, inserting the DNA into a suitable expression vector, transferring the vector with inserted DNA into an appropriate host, and expressing the polypeptide.

L10 ANSWER 5 OF 9 USPATFULL on STN
2000:43925 In vitro diagnostic assay employing HIV-2 antigens for the detection of HIV-2 specific antibodies.
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Geutard, Denise, Paris, France
Clavel, Francois, Rockville, MD, United States
Sonigo, Pierre, Paris, France
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Institut Pasteur, Paris, France (non-U.S. corporation)
US 6048685 20000411
APPLICATION: US 1995-466706 19950606 (8)
PRIORITY: FR 1986-911 19860122
FR 1986-1635 19860206
FR 1986-1985 19860213
FR 1986-3881 19860318
FR 1986-4215 19860324
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The molecular cloning and characterization of a novel human immunodeficiency virus type 2 (HIV-2), designated HIV-$2_{ROD}$, is described. A recombinant λ phage genomic library was screened with an HIV-2-specific probe to identify overlapping subgenomic clones. Fragments of these λ phage clones were subcloned into a suitable vector to reconstitute the complete HIV-$2_{ROD}$ genome. The complete nucleotide sequence of this proviral clone was ascertained and the following genes and gene products identified: gag (including p16, p26, and p12), pol, vif, **vpr**, vpx, env, tat, rev, and nef. These gene products will be useful, inter alia, in in vitro diagnostic methods and kits for the detection of HIV-2-specific antisera.
CLM What is claimed is:

1. An in vitro diagnostic method for detecting human immunodeficiency virus type 2 (HIV-2)-specific antisera comprising the following steps: (a) contacting a biological sample with one or more purified HIV-2 polypeptides selected from the group consisting of p16, p26, p12, Pol, Vif, **Vpr**, Vpx, Env, Tat, Rev, and Nef; (b) allowing said polypeptide to form an immune complex with HIV-2-specific antisera present in said sample; and, (c) detecting the formation of said immune complex.

2. The method of claim 1 wherein said immune complex is detected by a process selected from the group consisting of radioimmunoassay (RIA), radioimmunoprecipitation assay (RIPA), immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), and Western blot.

3. An in vitro diagnostic kit for detecting human immunodeficiency virus type 2 (HIV-2)-specific antisera comprising the following components: (a) one or more purified HIV-2 polypeptides selected from the group consisting of p16, p26, p12, Pol, Vif, **Vpr**, Vpx, Env, Tat, Rev, and Nef; (b) reagents for detecting the formation of an immune complex between said polypeptide and HIV-2-specific antisera present in a biological sample; and (c) a biological reference sample lacking antibodies that are recognized by said polypeptide.

L10 ANSWER 6 OF 9 USPATFULL on STN

1998:88668 Nucleotide sequences derived from the genome or retroviruses of the HIV-1, HIV-2, and SIV type, and their uses in particular for the amplification of the genomes of these retroviruses and for the in vitro diagnosis of the diseases due to these viruses.

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US 5786177 19980728

APPLICATION: US 1997-895231 19970716 (8)

PRIORITY: FR 1989-7954 19890602

FR 1989-12371 19890920

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to nucleotidic sequences derived from genomes of the HIV-1 type virus, or from genomes of the HIV-2 type virus, or of the SIV type virus, and their applications, especially as oligonucleotidic initiators of implementation of an (in vitro) method for the diagnosis of the infection of an individual by a virus of the HIV-1 and/or HIV-2 type.

CLM What is claimed is:

1. A method for the preparation of a polypeptide encoded by a region of the HIV or SIV genome, said method comprising: a) amplifying the nucleic acid encoding said polypeptide with at least two primers, wherein said first primer is complementary to a region of nucleotides of the nucleic acid of said genome, said second primer is complementary to a region of nucleotides of the strand of DNA complementary to said nucleic acid of said genome, wherein said regions of nucleotides are separated by about 100 to about 1100 base pairs when said complementary strands are hybridized to form one double-stranded nucleic acid, and said primer is selected from the group consisting of: nucleotides 900-881, 1385-1369, 1388-1369, and 2039-2021 of a nucleic acid sequence complementary to the gag gene of HIV-1 Bru; nucleotides 916-897, 1419-1403, 1421-1403, and 2073-2055 of a nucleic acid sequence complementary to the gag gene of HIV-1 Mal; nucleotides 900-881, 1385-1369, 1388-1369, and 2042-2024 of a nucleic acid sequence complementary the gag gene of HIV-1 Eli; nucleotides 1212-1193, 1703-1687, 1706-1687, and 2349-2329 of a nucleic acid sequence complementary to the gag gene of HIV-2 ROD; nucleotides 1176-1157, 1667-1651, 1670-1651, and 2381-2299 of a nucleic acid sequence complementary to the gag gene of SIV-MAC; nucleotides 5870-5849 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Bru; nucleotides 5865-5844 of a nucleic acid sequence complementary to the

vpr gene of HIV-1 Mal; nucleotides 6551-6531 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Eli; nucleotides 6551-6531 of a nucleic acid sequence complementary to the **vpr** gene of HIV-2 ROD; nucleotides 6454-6431 of a nucleic acid sequence complementary to the **vpr** gene of SIV-MAC; nucleotides 2643-2620, 3361-3339, 4207-4186, and 5011-4992 of a nucleic acid sequence complementary to the pol gene of HIV-1 Bru; nucleotides 2638-2615, 3356-3334, 4202-4181, and 5006-4987 of a nucleic acid sequence complementary to the pol gene of HIV-1 Mal; nucleotides 2607-2584, 3325-3303, 4171-4150, and 4975-4956 of a nucleic acid sequence complementary to the pol gene of HIV-1 Eli; nucleotides 2994-2971, 3712-3690, 4555-4534, and 5359-5340 of a nucleic acid sequence complementary to the pol gene of HIV-2 ROD; nucleotides 3010-2887, 3628-3606, 4471-4450, and 5275-5256 of a nucleic acid sequence complementary to the pol gene of SIV-MAC; nucleotides 9564-9542 and 9956-9933 of a nucleic acid sequence complementary to the nef2 gene of HIV-2 ROD; nucleotides 9538-9516 and 9839-9870 of a nucleic acid sequence complementary to the nef2 gene of SIV-MAC; nucleotides 5775-5754 and 6082-6061 of a nucleic acid sequence complementary to the vif2 gene of HIV-2 ROD; nucleotides 5691-5670 and 5995-5974 of a nucleic acid sequence complementary to the vif2 gene of SIV-MAC nucleotides 6228-6208 of a nucleic acid sequence complementary to the vpx gene of HIV-2 ROD; nucleotides 6141-6121 of a nucleic acid sequence complementary to the vpx gene of SIV-MAC; nucleotides 6930-6905, 7384-7360, 7857-7832, 8869-8844, and nucleotides 8242-8224 of a nucleic acid sequence complementary to the env gene of HIV-1 Bru; nucleotides 6928-6903, 7373-7349, 7846-7821, 8861-8836, and 8231-8213 of a nucleic acid sequence complementary to the env gene of HIV-1 Mal; nucleotides 6885-6860, 7330-7306, 7800-7775, 8812-8787, and 8185-8167 of a nucleic acid sequence complementary to the env gene of HIV-1 Eli; nucleotides 9136-9116 and 9503-9483 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Bru; nucleotides 9137-9117 and 9505-9484 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Mal; nucleotides 9082-9062 and 9449-9428 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Eli; nucleotides 5405-5383 and 5675-5653 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Bru; nucleotides 5400-5378 and 5670-5648 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Mal and nucleotides 5369-5347 and 5639-5617 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Eli; nucleotides 6343-6321 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Bru; nucleotides 6338-6316 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Mal; and nucleotides 6307-6285 of a nucleic acid sequence complementary to the vpu gene of SIV-MAC; b) introducing said amplified nucleotide sequence into a vector; c) transforming a host cell with said vector; and d) placing said transformed host cell in culture and recovering said polypeptide from said culture.

2. A method for the preparation of a polypeptide encoded by a region of the HIV or SIV genome, said method comprising: a) amplifying the nucleic acid encoding said polypeptide with at least two primers, wherein said first primer is complementary to a region of nucleotides of the nucleic acid of said genome, said second primer is complementary to a region of nucleotides of the strand of DNA complementary to said nucleic acid of said genome, wherein said regions of nucleotides are separated by about 100 to about 1100 base pairs when said complementary strands are hybridized to form one double-stranded nucleic acid, and said primer is selected from the group consisting of:

---

MMyl: TGG
CGC
CCG
AAC
AGG
GAC
TGG

CTG
AAC
AGG
GAC
MMy2: GGC
CAG
GGG
GAA
AGA
AAA
A
GGC
CCG
GCG
GAA
AGA
AAA
A
GGC
CCG
GAG
GAA
AGA
AAA
A
MMy3: TGC
CCA
TAC
AAA
ATG
TTT
TA
TGC
CCA
CAC
TAT
ATG
TTT
TA
MMy4: TGC
ATG
GCT
GCT
TGA
TG
TGC
ATA
GCT
GCC
TGG
TG
MMy4B:
CTT
TGC
ATG
GCT
GCT
TGA
TG
CTC
TGC
ATA
GCT
GCT
TGC

MMy4Ba:

CAT
CAA
GCA
GCC
ATG
CAA
AG

CAC
CAG
GCA
GCT
ATG
CAG
AG

MMy28:

AGG
GCT
GTT
GGA
AAT
GTG
G

AGG
GCT
GTT
GGA
AGT
GTG
G

MMy28a:

CCA
CAT
TTC
CAG
CAT
CCC
T

CCA
CAT
TTC
CAG
CAG
CCC
T

CCA
CAT
TTC
CAG
CAC
CCC
T

MMy18:

GAT
AGA
TGG
AAC
AAG
CCC
CAG

MMy19:

TCC
ATT
TCT
TGC

CCT
CTG
T

MMy29:

TAA
AGC
CAG
GAA
TGG
ATG
GCC
CAA

TAA
AGC
CAG
GAA
TGG
ATG
GAC
CAA

MMy29a:

TTG
GGC
CAT
CCA
TTC
CTG
GCT
TTA

TTG
GTC
CAT
CCA
TTC
CTG
GCT
TTA

MMy30:

TGG
ACT
GTC
AAT
GAC
ATA
CAG
AA

TGG
ACT
GTC
AAT
GAT
ATA
CAG
AA

MMy30a:

TTC
TGT
ATG
TCA
TTG
ACA
GTC
CA

TTC
TGT

TCA
TTG
ACT
GTC
CA

MMy31:

CAT
GGG
TAC
CAG
CAC
ACA
AAG
G

MMy31a:

CCT
TTG
TGT
GCT
GGT
ACC
CAT
G

MMy32:

TGG
AAA
GGT
GAA
GGG
GCA
GT

TGG
AAA
GGT
GAA
GGA
GCA
GT

MMy32a:

ACT
GCC
CCT
TCA
CCT
TTC
CA

ACT
GCC
CCT
TCT
CCT
TTC
CA

ACT
GCC
CCT
TCC
CCT
TTC
CA

MMy12:

AGA
GAC
TCT
TGC

CGC
GTG

MMy13:

ATA
TAC
TTA
GAA
AAG
GAA
GAA
GG

MMy13a:

CCT
TCT
TCC
TTT
TCT
AAG
TAT
AT

MMy14:

AGC
TGA
GAC
AGC
AGG
GAC
TTT
CCA

MMy20:

TAT
GGA
GGA
GGA
AAA
GAG
ATG
GAT
AGT

MMy21:

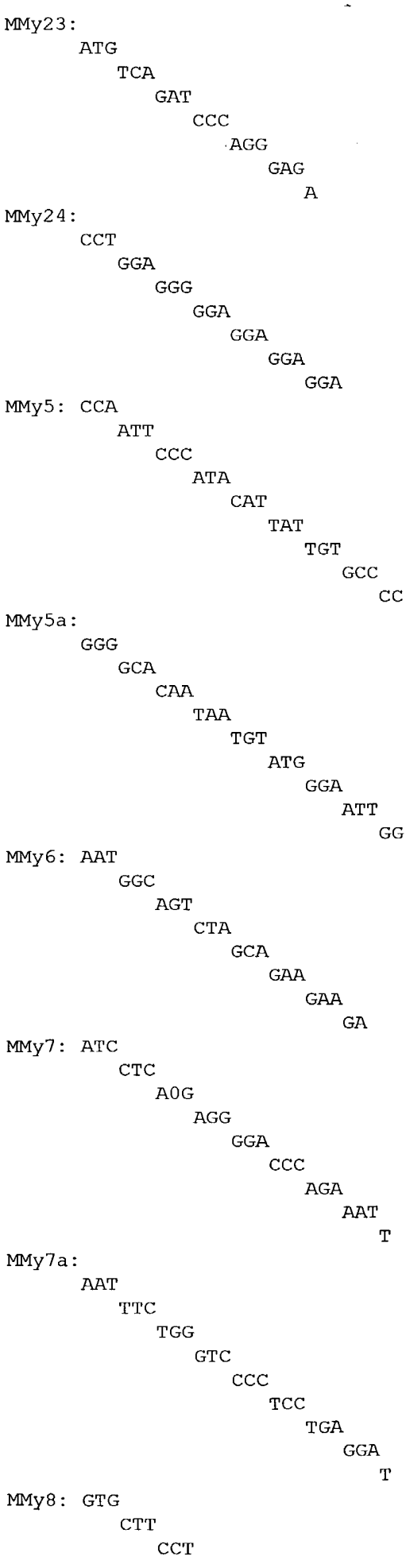
TAG
CAC
TTA
TTT
CCC
TTG
CTT
T

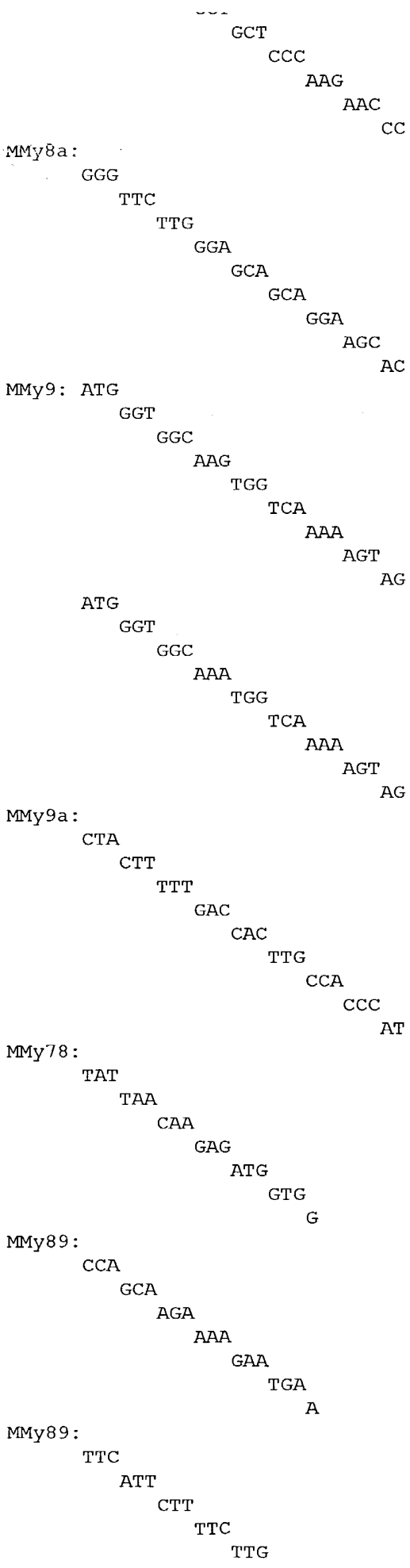
MMy21a:

AAA
GCA
AGG
GAA
ATA
AGT
GCT
A

MMY22:

CCC
TTG
TTC
ATC
ATG
CCA
GTA


MMy23:
ATG
TCA
GAT
CCC
AGG
GAG
A
MMy24:
CCT
GGA
GGG
GGA
GGA
GGA
GGA
MMy5: CCA
ATT
CCC
ATA
CAT
TAT
TGT
GCC
CC
MMy5a:
GGG
GCA
CAA
TAA
TGT
ATG
GGA
ATT
GG
MMy6: AAT
GGC
AGT
CTA
GCA
GAA
GAA
GA
MMy7: ATC
CTC
A0G
AGG
GGA
CCC
AGA
AAT
T
MMy7a:
AAT
TTC
TGG
GTC
CCC
TCC
TGA
GGA
T
MMy8: GTG
CTT
CCT


GCT
CCC
AAG
AAC
CC
MMy8a:
GGG
TTC
TTG
GGA
GCA
GCA
GGA
AGC
AC
MMy9: ATG
GGT
GGC
AAG
TGG
TCA
AAA
AGT
AG
ATG
GGT
GGC
AAA
TGG
TCA
AAA
AGT
AG
MMy9a:
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CTT
TTT
GAC
CAC
TTG
CCA
CCC
AT
MMy78:
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TAA
CAA
GAG
ATG
GTG
G
MMy89:
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AGA
AAA
GAA
TGA
A
MMy89:
TTC
ATT
CTT
TTC
TTG

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         AGA
            AAA
               GGG
                  GGG
                     ACT
                        GGA
MMy10a:
      TCC
         AGT
            CCC
               CCC
                  TTT
                     TCT
                        TTT
MMy11:
      AAA
         GTC
            CCC
               AGC
                  GGA
                     AAG
                        TCC
                           C
MMy15:
      GAT
         TAT
            GGA
               AAA
                  CAG
                     ATG
                        GCA
                           GGT
                              GAT
MMy16:
      GCA
         GAC
            CAA
               CTA
                  ATT
                     CAT
                        CTG
                           TA
MMy16a:
      TAC
         AGA
            TGA
               ATT
                  AGT
                     TGG
                        TCT
                           GC
MMy17:
      CTT
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            CTC
               CTC
                  TAA
                     AAG
                        CTC
                           TA
MMy25:
      GTA
```

AGT
ACA
TGT
AAT
GCA
ACC
T

MMy26:
AGC
AGA
AGA
CAG
TGG
CCA
TGA
GAG

MMy27:
ACT
ACA
GAT
CAT
CAA
TAT
CCC
AA

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b) introducing said amplified nucleotide sequence into a vector; c) transforming a host cell with said vector; and d) placing said transformed host cell in culture and recovering said polypeptide from said culture.

L10 ANSWER 7 OF 9 USPATFULL on STN
1998:75745 DNA fragments obtained from a novel human immunodeficiency virus designated $LAV_{MAL}$.
Alizon, Marc, Paris, France
Sonigo, Pierre, Paris, France
Wain-Hobson, Simon, Montigny les Bretonneux, France
**Montagnier, Luc**, Le Plessis Robinson, France
Institut Pasteur, Paris, France (non-U.S. corporation)
US 5773602 19980630
APPLICATION: US 1993-154397 19931118 (8)
PRIORITY: FR 1986-401380 19860623
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A novel human immunodeficiency virus type 1 (HIV-1) isolate, designated lymphadenopathy-associated virus strain MAL, or $LAV_{MAL}$, was molecularly cloned and characterized. Nucleotide sequence analysis demonstrated that the viral genome of $LAV_{MAL}$ is 9229 nucleotides long. This retrovirus contains the canonical gag, pol, and env genes, as well as ancillary genes encoding Vif (or Q), **Vpr** (or R), Tat (or S), and Nef (or F). This virus differs significantly, at both the nucleotide and amino acid sequence levels, from prototypical HIV isolates (e.g., HTLV-III, $LAV_{BRU}$, and ARV). DNA fragments corresponding to the various gene products and regulatory regions are disclosed. These fragments are useful, inter alia, as probes in diagnostic assays and for the generation of recombinant proteins.
CLM What is claimed is:
1. A DNA fragment having a nucleotide sequence selected from the group consisting of: a sequence having nucleotides 1 to 96, which is the long terminal repeat R region of $LAV_{MAL}$ ; a sequence having nucleotides 97 to 179, which is the 5' long terminal repeat U5 region of $LAV_{MAL}$ ; a sequence having nucleotides 8676 to 9133, which is the 3' long

terminal repeat U5 region of $LAV_{MAL}$ ; a sequence having nucleotides 9134 to 9229, which is the 3' long terminal repeat U3 region of $LAV_{MAL}$ ; a sequence having nucleotides 5405 to 5620, which is the tat coding region of $LAV_{MAL}$ ; a sequence having nucleotides 5134 to 5421, which is the **vpr** coding region of $LAV_{MAL}$ ; a sequence having nucleotides 8380 to 9006, which is the nef coding region of $LAV_{MAL}$ ; a sequence having nucleotides 350 to 1864, which is the gag coding region of $LAV_{MAL}$ ; a sequence having nucleotides 1663 to 4668, which is the pol coding region of $LAV_{MAL}$ ; a sequence having nucleotides 5799 to 8375, which is the env coding region of $LAV_{MAL}$ ; a sequence having nucleotides 764 to 1501, which is the gag p25 coding region of $LAV_{MAL}$ ; a sequence having nucleotides 1502 to 1864, which is the gag p13 coding region of $LAV_{MAL}$ ; a sequence having nucleotides 5799 to 5885, which corresponds to amino acids 1-33 of the env coding region of $LAV_{MAL}$ ; a sequence having nucleotides 5886 to 7337, which corresponds to amino acids 34 to 530 of the gp110 env coding region of $LAV_{MAL}$ ; a sequence having nucleotides 5895 to 6176, which corresponds to amino acids 37 to 130 of the env coding region of $LAV_{MAL}$ ; a sequence having nucleotides 6399 to 6635, which corresponds to amino acids 211 to 289 of the env coding region of $LAV_{MAL}$ ; a sequence having nucleotides 7212 to 7337, which corresponds to amino acids 488 to 530 of the env coding region of $LAV_{MAL}$ ; a sequence having nucleotides 7215 to 7604, which corresponds to amino acids 490 to 620 of the env coding region of $LAV_{MAL}$ ; and a sequence having nucleotides 7782 to 7844, which corresponds to amino acids 680 to 700 of the env coding region of $LAV_{MAL}$.

2. The DNA fragment as claimed in claim 1, wherein said fragment is operatively linked to a promoter sequence.

3. A DNA fragment as claimed in claim 1, wherein said fragment has a nucleotide sequence having nucleotides 1 to 96, which is the long terminal repeat R region of $LAV_{MAL}$.

4. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 97 to 179, which is the 5' long terminal repeat U5 region of $LAV_{MAL}$.

5. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 8676 to 9133, which is the 3' long terminal repeat U3 region of $LAV_{MAL}$.

6. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 9134 to 9229, which is the 3' long terminal repeat U3 region of $LAV_{MAL}$.

7. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 5405 to 5620, which is the tat coding region of $LAV_{MAL}$.

8. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 5134 to 5421, which is the **vpr** coding region of $LAV_{MAL}$.

9. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 8380 to 9006, which is the nef coding region of $LAV_{MAL}$.

10. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 350 to 1864, which is the gag coding region of $LAV_{MAL}$.

11. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 1663 to 4668, which is the pol coding region of $LAV_{MAL}$.

12. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 5799 to 8375, which is the env coding region of $LAV_{MAL}$.

13. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 764 to 1501, which is the gag p25 coding region of $LAV_{MAL}$.

14. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 1502 to 1864, which is the gag p13 coding region of $LAV_{MAL}$.

15. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 5799 to 5885, which corresponds to amino acids 1-33 of the env coding region of $LAV_{MAL}$.

16. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 5886 to 7337, which corresponds to amino acids 34 to 530 of the gp110 env coding region of $LAV_{MAL}$.

17. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 5895 to 6176, which corresponds to amino acids 37 to 130 of the env coding region of $LAV_{MAL}$.

18. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 6399 to 6635, which corresponds to amino acids 211 to 289 of the env coding region of $LAV_{MAL}$.

19. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 7212 to 7337, which corresponds to amino acids 488 to 530 of the env coding region of $LAV_{MAL}$.

20. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 7215 to 7604, which corresponds to amino acids 490 to 620 of the env coding region of $LAV_{MAL}$.

21. A DNA fragment as claimed in claim 1, wherein said fragment has a sequence having nucleotides 7782 to 7844, which corresponds to amino acids 680 to 700 of the env coding region of $LAV_{MAL}$.

22. A recombinant vector comprising a DNA fragment of any one of claims 1-21.

23. A transformed host comprising the recombinant vector of claim 22.

L10 ANSWER 8 OF 9 USPATFULL on STN
97:106928 Nucleotide sequences derived from the genome of retroviruses of the HIV-1, HIV-2 and SIV type, and their uses in particular for the amplification of the genomes of these retroviruses and for the in vitro diagnosis of the disease due to these viruses.
Moncany, Maurice, Paris, France
**Montagnier, Luc**, Le Plessis-Robinson, France
Institut Pasteur, France (non-U.S. corporation) Institut National de la Sante et de la Recherche Medicale, France (non-U.S. corporation)
US 5688637 19971118
APPLICATION: US 1993-160465 19931202 (8)
PRIORITY: FR 1989-7354 19890602
FR 1989-12371 19890920

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to nucleotidic sequences derived from genomes of the HIV-1 type virus, or from genomes of the HIV-2 type virus, or of the SIV type virus, and their applications, especially as oligo-nucleotidic initiators of implementation of an $i (in vitro) method for the diagnosis of the infection of an individual by a virus of the HIV-1 and/or HIV-2 type.

CLM What is claimed is:

1. An oligonucleotide primer, said primer having a nucleotide sequence selected from the following group of nucleotides oriented in the 5'-3' direction: nucleotides 636-653, 854-872, 1369-1388, and 2021-2039 of the gag gene of HIV-1 Bru; nucleotides 900-881, 1385-1369, 1388-1369, and 2039-2021 of a nucleic acid sequence complementary to the gag gene of HIV-1 Bru; nucleotides 635-652, 864-888, 1403-1421, and 2055-2073 of the gag gene of HIV-1 Mal; nucleotides 916-897, 1419-1403, 1421-1403, and 2073-2055 of a nucleic acid sequence complementary to the gag gene of HIV-1 Mal; nucleotides 636-653, 848-872, 1369-1388, and 2024-2042 of the gag gene of HIV-1 Eli; nucleotides 900-881, 1385-1369, 1388-1369, and 2042-2024 of a nucleic acid sequence complementary to the gag gene of HIV-1 Eli; nucleotides 859-876, 1160-1184, 1687-1706, and 2329-2349 of the gag gene of HIV-2 ROD; nucleotides 1212-1193, 1703-1687, 1706-1687, and 2349-2329 of a nucleic acid sequence complementary to the gag gene of HIV-2 ROD; nucleotides 834-851, 1124-1148, 1651-1670, and 2299-2318 of the gag gene of SIV-MAC; and nucleotides 1176-1157, 1667-1651, 1670-1651, and 2318-2299 of a nucleic acid sequence complementary to the gag gene of SIV-MAC; nucleotides 5590-5610 of the **vpr** gene of HIV-1 Bru; nucleotides 5870-5849 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Bru; nucleotides 5585-5605 of the **vpr** gene of HIV-1 Mal; nucleotides 5865-5844 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Mal, nucleotides 5554-5574 of the **vpr** gene of HIV-1 Eli; nucleotides 5834-5813 of a nucleic acid sequence complementary to the **vpr** gene of HIV-1 Eli; nucleotides 6233-6296 of the **vpr** gene of HIV-2 ROD; nucleotides 6551-6531 of a nucleic acid sequence complementary to the **vpr** gene of HIV-2 ROD; nucleotides 6147-6170 of the **vpr** gene of SIV-MAC; and nucleotides 6454-6431 of a nucleic acid sequence complementary to the **vpr** gene of SIV-MAC; nucleotides 2620-2643, 3339-3361, 4186-4207, and 4992-5011 of the pol gene of HIV-1 Bru; nucleotides 2643-2620, 3361-3339, 4207-4186, and 5011-4992 of a nucleic acid sequence complementary to the pol gene of HIV-1 Bru; nucleotides 2615-2638, 3333-3356, 4181-4202, and 4987-5006 of the pol gene of HIV-1 Mal; nucleotides 2638-2615, 3356-3334, 4202-4181, and 5006-4987 of a nucleic acid sequence complementary to the pol gene of HIV-1 Mal; nucleotides 2584-2607, 3303-3325, 4150-4171, and 4956-4975 of the pol gene of HIV-1 Eli; nucleotides 2607-2584, 3325-3303, 4171-4150, and 4975-4956 of a nucleic acid sequence complementary to the pol gene of HIV-1 Eli; nucleotides 2971-2994, 3690-3712, 4534-4555, and 5340-5359 of the pol gene of HIV-2 ROD; nucleotides 2994-2971, 3712-3690, 4555-4534, and 5359-5340 of a nucleic acid sequence complementary to the pol gene of HIV-2 ROD; nucleotides 2887-3010, 3606-3628, 4450-4471, and 5275-5256 of a nucleic acid sequence complementory to the pol gene of SIV-MAC; 5256-5275 of the pol gene of SIV-MAC; and nucleotides 3010-2887, 3628-3606, 4471-4450, and nucleotides 9165-9185 and 9542-9564 of the nef2 gene of HIV-2 ROD; 9564-9542 and 9956-9933 of a nucleic acid sequence complementary to the nef2 gene of HIV-2 ROD; nucleotides 9139-9159 and 9516-9538 of the nef2 gene of SIV-MAC; 9538-9516 and 9839-9870 of a nucleic acid sequence complementary to the nef2 gene of SIV-MAC; nucleotides 5424-5450 and 5754-5775 of the vif2 gene of HIV-2 ROD; nucleotides 5775-5754 and 6082-6061 of a nucleic acid sequence complementary to the vif2 gene of HIV-2 ROD; nucleotides 5340-5366 and 5670-5691 of the vif2 gene of HIV-2 ROD; nucleotides 5691-5670 and 5995-5974 of a nucleic acid sequence complementary to the vif2 gene of SIV-MAC; nucleotides 5900-5918 of the vpx gene of HIV-2 ROD; nucleotides 6228-6208 of a nucleic acid sequence complementary to the vpx gene of HIV-2 ROD; nucleotides 5813-5831 of the vpx gene of HIV-2 ROD; nucleotides 6141-6121 of a nucleic acid sequence

complementary to the vpx gene of SIV-MAC, nucleotides 6906-6883, 7055-7077, 7360-7384, 7832-7857, 8844-8869, 7629-7647, and 8224-8242 of the env gene of HIV-1 Bru; nucleotides 6930-6905, 7384-7360, 7857-7832, 8869-8844, and 8242-8224 of a nucleic acid sequence complementary to the env gene of HIV-1 Bru; nucleotides 6903-6928, 7053-7075, 7821-7846, 7821-7846, 7612-7630, 8213-8231, and 8836-8861 of the env gene of HIV-1 Mal; nucleotides 6928-6903, 7373-7349, 7846-7821, 8861-8836, and 8231-8213 of a nucleic acid sequence complementary to the env gene of HIV-1 Mal; nucleotides 6860-6885, 7010-7032, 7306-7330, 7775-7800, 8787-8812, 7572-7590, and 8167-8185 of the env gene of HIV-1 Eli; and nucleotides 6885-6860, 7330-7306, 7800-7775, 8812-8787, and 8185-8167 of a nucleic acid sequence complementary to the env gene of HIV-1 Eli; nucleotides 9116-9136 of the nef1 gene of HIV-1 Bru; nucleotides 9136-9116 and 9503-9483 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Bru; nucleotides 9117-9137 of the nef1 gene of HIV-1 Mal; and nucleotides 9137-9117 and 9505-9484 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Mal; nucleotides 9062-9082 of the nef1 gene of HIV-Eli; nucleotides 9082-9062 and 9449-9428 of a nucleic acid sequence complementary to the nef1 gene of HIV-1 Eli; nucleotides 5073-5099 and 5383-5405 of the vif1 gene of HIV-1 Bru; and nucleotides 5405-5383 and 5675-5653 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Bru; nucleotides 5068-5094 and 5378-5400 of the vif1 gene of HIV-1 Mal; nucleotides 5400-5378 and 5670-5648 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Mal; and nucleotides 5037-5063 and 5347-5369 of the vif1 gene of HIV-1 Eli; nucleotides 5369-5347 and 5639-5617 of a nucleic acid sequence complementary to the vif1 gene of HIV-1 Eli; nucleotides 6081-6105 and 6240-6263 of the vpu gene of HIV-1 Bru; nucleotides 6343-6321 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Bru; nucleotides 6076-6100 and 6238-6261 of the vpu gene of HIV-1 Mal; nucleotides 6338-6316 of a nucleic acid sequence complementary to the vpu gene of HIV-1 Mal; nucleotides 6045-6069 and 6207-6230 of the vpu gene of SIV-MAC; and nucleotides 6307-6285 of a nucleic acid sequence complementary to the vpu gene of SIV-MAC.

2. An oligonucleotide primer selected from the group consisting of primers having the following nucleotide sequences from 5' to 3': MMy1: TGG CGC CCGAAC AGG GAC TGG CGC CTGAAC AGG GAC MMy2: GGC CAG GGG GAAAGAAAAA GGC CCG GCG GAAAGAAAAA MMy3: TGC CCA TACAAAATG TTT TA TGC CCA CAC TAT ATG TTT TA MMy4: TGC ATG GCT GCT TGA TG TGC ATA GCT GCC TGG TG MMy4B: CTT TGC ATG GCT GCT TGA TG CTC TGC ATA GCT GCT TGC TG MMy4Ba: CAT CAAGCA GCC ATG CAAAG CAC CAG GCA GCT ATG CAG AG MMy28: AGG GCT GTT GGAAAT GTG G AGG GCT GTT GGA AGT GTG G MMy28a: CCA CAT TTC CAG CAT CCC T CCA CAT TTC CAG CAG CCC T CCA CAT TTC CAG CAC CCC T MMy18: GAT AGA TGGAAC AAG CCC CAG MMy19: TCC ATT TCT TGC TCT CCT CTG T MMy29: TAAAGC CAG GAA TGG ATG GCC CAA TAAAGC CAG GAA TGG ATG GAC CAA MMy29a: TTG GGC CAT CCA TTC CTG GCT TTA TTG GTC CAT CCA TTC CTG GCT TTA MMy30: TGG ACT GTC AAT GAC ATA CAGAA TGG ACT GTC AAT GAT ATA CAGAA MMy30a: TTC TGT ATG TCA TTG ACA GTC CA TTC TGT ATG TCA TTG ACT GTC CA MMy31: CAT GGG TAC CAG CAC ACAAAG G MMy31a: CCT TTG TGT GCT GGT ACC CAT G MMy32: TGG AAA GGT GAA GGG GCA GT TGG AAA GGT GAAGGA GCA GT MMy32a: ACT GCC CCT TCA CCT TTC CA ACT GCC CCT TCT CCT TTC CA ACT GCC CCT TCC CCT TTC CA MMy12: AGA GAC TCT TGC GGG CGC GTG MMy13: ATA TAC TTA GAAAAG GAA GAAGG MMy13a: CCT TCT TCC TTT TCTAAG TAT AT MMy14: AGC TGA GAC AGC AGG GAC TTT CCA MMy20: TAT GGA GGA GGAAAAGAG ATG GAT AGT MMy21: TAG CAC TTA TTT CCC TTG CTT T MMy21a: AAA GCA AGG GAAATA AGT GCT A MMY22: CCC TTG TTC ATC ATG CCA GTA T MMy23: ATG TCA GAT CCC AGG GAG A MMy24: CCT GGA GGG GGA GGA GGA GGA MMy5: CCA ATT CCC ATA CAT TAT TGT GCC CC MMy5a: GGG GCA CAA TAATGT ATG GGA ATT GG MMy6: AAT GGC AGT CTA GCA GAA GAA GA MMy7: ATC CTC A0G AGG GGA CCC AGAAAT T MMy7a: AAT TTC TGG GTC CCC TCC TGA GGA T MMy8a: GTG CTT CCT GCT GCT CCC AAG AAC CC MMy8a: GGG TTC TTG GGA GCA GCA GGA AGC AC MMy9: ATG GGT GGC AAG TGG TCAAAAAGT AG ATG GGT GGCAAATGG TCAAAAAGT AG MMy9a: CTA CTT TTT GAC CAC TTG CCA CCC AT MMy89: TTC ATT CTT TTC TTG CTG G MMy10: AAAAGAAAAGGG GGG ACT GGA MMy10a: TCC AGT CCC CCC TTT TCT TTT MMy11: AAA GTC CCC AGC GGAAAG TCC C MMy15: GAT TAT GGAAAA CAG ATG GCA GGT GAT MMy16: GCAGAC CAACTA ATT CAT CTG TA MMy16a:

MMy25: GTA AGT AGT ACA TGTAAT GCA ACC T MMy26: AGC AGA AGA CAG TGG CCATGA GAG MMy27: ACT ACA GAT CAT CAATAT CCC AA.

3. A method for amplifying nucleic acids of viruses of the HIV-1, HIV-2, and SIV type in a biological sample, said method comprising a) extracting said nucleic acid from said biological sample; b) treating said nucleic acid with a reverse transcriptase if said nucleic acid is RNA; and c) performing an amplification cycle comprising the following steps: denaturing the nucleic acid to be detected to form single-stranded nucleic acids, hybridizing each of said nucleic acid single strands with at least one primer according to any one of claims 1 and 2, by placing said single strands in contact with at least one of said primers, and amplifying said nucleic acid strands by elongation of said primers along the strands to which they are hybridized in the presence of a polymerase, dATP, dGTP, dCTP and dTTP, said cycle being repeated about 30 to about 40 times.

4. The method of claim 3 wherein the step of denaturing the nucleic acid is carried out in the presence of said primer.

5. A method of in vitro diagnosis of infection of a mammal by a virus selected from the group consisting of HIV-1, HIV-2, and SIV, said method comprising detecting nucleic acid of said virus by a) obtaining a biological sample from said mammal, wherein said biological sample comprises nucleic acid; b) extracting nucleic acid of said virus from said biological sample and, if said nucleic acid is RNA, treating said nucleic acid with a reverse transcriptase to produce a double-stranded nucleic acid comprising said nucleic acid and its complementary strand; c) performing an amplification cycle comprising the following steps: denaturing the double-stranded nucleic acid to be detected to form single-stranded nucleic acids, hybridizing each of said nucleic acid single strands with at least one primer according to any one of claims 1 and 2, by placing said single strand in contact with said primer under hybridization conditions, and amplifying said nucleic acid single strands by elongation of said primers along the strands to which they are hybridized in the presence of a polymerase, dATP, dGTP, dCTP and dTTP, said cycle being repeated about 10 to about 60 times; d) detecting the nucleic acid of said virus and e) correlating the presence of the nucleic acid of said virus with infection by said virus.

6. The diagnostic method of claim 5, wherein the hybridization step of the cycle is carried out by placing each of said single-stranded nucleic acids in contact with said primers, wherein said primers hybridize with a nucleotide sequence situated on the first strand of said double-stranded nucleic acid and with a nucleotide sequence situated on the strand complementary to said first strand, said nucleic acid sequences being separated by a region of about 50 to about 10,000 base pairs when said complementary strands are hybridized to form one double-stranded nucleic acid.

7. The method of claim 6, wherein said region is about 100 to about 1100 base pairs.

8. The method according to claim 5, wherein said detecting step (d) comprises hybridizing at least one detectably labelled nucleotide probe to said amplified nucleic acid.

9. The method of claim 5 wherein said virus is HIV-1 or HIV-2, and said primer couple is selected from the group consisting of MMy1-MMy4, MMy2-MMy4, MMy1-MMy3, MMy18-MMy19, MMy4a-MMy28a, MMy28-MMy29a, MMy29-MMy30a, and MMy31-MMy32a.

10. The method of claim 5 wherein said virus is HIV-1, and said primer couple is selected from the group consisting of MMy5-MMy8, MMy6-MMy8, MMy7-MMy8, MMy5-MMy7a, MMy6-MMy7a, MMy9-MMy11, MMy10-MMy11, MMy9-MMy10a,

MMy15-MMy16a, MMy16-MMy17, MMy25-MMy27, and MMy26-MMy27.

11. The method of claim 5, wherein said virus is HIV-2, and said primer, couple is selected from the group consisting of MMy20-MMy22, MMy20,-MMy21a, MMy21-MMy22, MMy23-MMy24, MMy12-MMy14, and MMy12 MMy13a.

12. The method of claim 5, wherein said virus comprises a gene selected from the group consisting of gag, **vpr**, env, nef1, vif1, vif2, vpx, nef2, vpu and pol, and said primer couple is selected from the group consisting of MMy1-MMy4, MMy2-MMy4, MMy1-MMy3, MMy4a-MMy28a for the gag gene; MMy18-MMy19 for the **vpr** gene; MMy5-MMy8, MMy6-MMy8, MMy7-MMy8, MMy5-MMy7a, MMy6-MMy7a, MMy26-MMy5a, MMy8a-MMy9a, MMy8a-MMy89, MMy89a-MMy9a for the env gene; MMy9-MMy11, MMy9-MMy10a, MMy10-MMy11 for the nef1 gene; MMy15-MMy17, MMy15-MMy16a, MMy16-MMy17 for the vif1 gene; MMy20-MMy22, MMy20-MMy21a, MMy21-MMy22 for the vif2 gene; MMy23-MMy24 for the vpx gene; MMy12-MMy14, MMy12-MMy13a, MMy13-MMy14 for the nef2 gene; MMy25-MMy27, MMy26-MMy27 for the vpu gene; and MMy28-MMy29a, MMy29-MMy30a, MMy30-MMy31a, MMy31-MMy32a for the pol gene.

13. A diagnostic kit for the in vitro diagnosis of infection of a meal by a virus selected from the group consisting of HIV-1, HIV-2, and SIV by detecting the presence of HIV-1, HIV-2 or SIV nucleic acid or a strand of DNA complementary to said nucleic acid, said kit comprising a) at least a first and a second prime according to any one of claims 1 and 2, wherein said first primer is complementary to a region of nucleotides of the nucleic acid of said virus, and said second primer is complementary to a region of nucleotides of the strand of DNA complementary to said nucleic acid of said virus, wherein said regions of nucleotides are separated by about 50 to about 10,000 base pairs when said complementary strands are incorporated into one double-stranded nucleic acid; b) reagents for amplifying said nucleic acid; and c) at least one detectably labelled probe capable of hybridizing with the amplified nucleotide sequence to be detected.

14. An oligonucleotide primer couple for the amplification according to any one of claims 3 and 5, said primer couple selected from the group consisting of MMy4Ba-MMy28a, MMy26-MMy5a, MMy8a-MMy89, MMy89a-MMy9a, MMy25-MMy27, MMy26-MMy27, MMy28-MMy29a, MMy29-MM30a, MMy30-MMy31a, and MMy31-MMy32a.

L10 ANSWER 9 OF 9 USPATFULL on STN
96:111330 Peptides of human immunodeficiency virus type 2 (HIV-2) and in vitro diagnostic methods and kits employing the peptides for the detection of HIV-2.
Alizon, Marc, Paris, France
**Montagnier, Luc**, Le Plessis Robinson, France
Geutard, Denise, Paris, France
Clavel, Francois, Rockville, MD, United States
Sonigo, Pierre, Paris, France
Guyader, Mireille, Paris, France
Institut Pasteur, Paris, France (non-U.S. corporation)
US 5580739 19961203
APPLICATION: US 1994-214221 19940317 (8)
PRIORITY: FR 1986-911 19860122
FR 1986-1635 19860206
FR 1986-1985 19860213
FR 1986-3881 19860318
FR 1986-4215 19860324
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A novel lentivirus, designated the human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), was isolated from West African patients with acquired immune deficiency syndrome (AIDS). A recombinant lambda phage library was constructed from $HIV\text{-}2_{ROD}$ -infected CEM genomic DNA.

overlapping molecular clones were obtained and the nucleotide sequence of the complete 9.5-kilobase (kb) HIV-$2_{ROD}$ genome ascertained. The genetic organization of HIV-2 is analogous to that of other retroviruses and consists of the 5'LTR-gag-pol-central region-env-nef-3'LTR. The central region also encodes for the regulatory proteins Tat and Rev, as well as the ancillary proteins Vif, **Vpr**, and Vpx. The proteins encoded by this proviral clone will provide novel immunologic, biochemic, and diagnostic reagents useful for the detection of HIV-2.

CLM What is claimed is:

1. A peptide comprising the gag precursor protein of human immunodeficiency virus type 2 (HIV-$2_{ROD--}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR4##

2. A peptide comprising the polymerase precursor protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR5##

3. A peptide comprising the Vif protein of human immunodeficiency virus type 2 (HIV-$2_{ROD--}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR6##

4. A peptide comprising the **Vpr** protein of human immunodeficiency virus type 2 (HIV-$2_{ROD--}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR7##

5. A peptide comprising the Vpx protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR8##

6. A peptide comprising the Nef protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR9##

7. A peptide comprising the TAT protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR10##

8. A peptide comprising the Rev protein of human immunodeficiency virus type 2 (HIV-$2_{ROD--}$), wherein the peptide is free of particles of said virus, having the following amino acid sequence: ##STR11##

9. An in vitro diagnostic method for the detection of the presence or absence of antibodies which bind to antigens of a human immunodeficiency virus type 2 (HIV-2) comprising: (a) contacting a biological sample with one or more peptides selected from the group consisting of: (1) a peptide comprising the gag precursor protein of human immunodeficiency virus type 2 (HIV-$2_{ROD--}$), having the following sequence: ##STR12## (2) a peptide comprising the polymerase precursor protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the following sequence: ##STR13## (3) a peptide comprising the env precursor protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the following sequence: ##STR14## (4) a peptide comprising the Vif protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the following sequence: ##STR15## (5) a peptide comprising the **Vpr** protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the following sequence: ##STR16## (6) a peptide comprising the Vpx protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the following sequence: ##STR17## (7) a peptide comprising the Nef protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the following sequence: ##STR18## (8) a peptide comprising the TAT protein of human immunodeficiency virus type 2 (HIV-$2_{ROD}$), having the

following sequence: ##STR19## (9) a peptide comprising the Nef protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR20## (10) a peptide comprising the p16/matrix protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR21## (11) a peptide comprising the p26/capsid protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR22## (12) a peptide comprising the p12/nucleocapsid protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR23## (b) detecting the formation of antigen-antibody complex between said one or more peptides and antibodies present in said biological sample: and (c) providing a biological reference sample lacking antibodies recognized by said one or more peptides, wherein the one or more peptides and the biological reference sample are present in an amount sufficient to perform the detection of antigen-antibody complex formed between said one or more peptides and antibodies present in the biological sample, said detection being indicative of previous exposure to HIV-2.

10. The method of claim 9, wherein the formation of antigen-antibody complex is detected by employing a process selected from the group consisting of radioimmunoassay, radioimmunoprecipitation assay, immunofluorescence assay, enzyme-linked immunosorbent assay, and Western blot.

11. An in vitro diagnostic method for the detection of the presence or absence of antibodies which bind to antigens of a human immunodeficiency virus type 2 (HIV-2) comprising: (a) contacting a biological sample with a peptide comprising the Nef protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR24## (b) detecting the formation of antigen-antibody complex between said peptide and antibodies present in said biological sample; and (c) providing a biological reference sample lacking antibodies recognized by said peptide. wherein the peptide and the biological reference sample are present in an amount sufficient to perform the detection of antigen-antibody complex formed between said peptide and antibodies present in the biological sample, said detection being indicative of previous exposure to HIV-2.

12. The method of claim 11, wherein the formation of antigen-antibody complex is detected by employing a process selected from the group consisting of radioimmunoassay, radioimmunoprecipitation assay, immunofluorescence assay, enzyme-linked immunosorbent assay, and Western blot.

13. A diagnostic kit for the in vitro detection of the presence or absence of antibodies which bind to antigens of a human immunodeficiency virus type 2 (HIV-2) comprising: (a) a peptide composition comprising one or more peptides selected from the group consisting of: (1) a peptide comprising the gag precursor protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR25## (2) a peptide comprising the polymerase precursor protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR26## (3) a peptide comprising the env precursor protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR27## (4) a peptide comprising the Vif protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR28## (5) a peptide comprising the **Vpr** protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR29## (6) a peptide comprising the Vpx protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR30## (7) a peptide comprising the Nef protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR31## (8) a peptide comprising the TAT protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the

following sequence: ##STR32## (9) a peptide comprising the rev protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR33## (10) a peptide comprising the p16/matrix protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR34## (11) a peptide comprising the p26/capsid protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR35## (12) a peptide comprising the p12/nucleocapsid protein of human immunodeficiency virus type 2 ($HIV\text{-}2_{ROD}$), having the following sequence: ##STR36## (b) reagents for the detection of the formation of antigen-antibody complex; and (c) a biological reference sample lacking antibodies recognized by said peptide composition, wherein the peptide composition, reagents, and biological reference sample are present in an amount sufficient to perform the detection of antigen-antibody complex formed between said one or more peptides and antibodies present in the biological sample, said detection being indicative of previous exposure to HIV-2.

14. A diagnostic kit for the in vitro detection of the presence or absence of antibodies which bind to antigens of a human immunodeficiency virus type 2 (HIV-2) comprising: (a) a peptide having the following sequence: ##STR37## (b) reagents for the detection of the formation of antigen-antibody complex; and (c) a biological reference sample lacking antibodies recognized by said peptide; wherein the peptide, reagents, and biological reference sample are present in an amount sufficient to perform the detection of antigen-antibody complex formed between said peptide and antibodies present in the biological sample, said detection being indicative of previous exposure to HIV-2.

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=> d his

     (FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
                E WEINER DAVID/IN
L1            40 S E5
L2            49 S E3 OR E5
L3            26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4            26 S L3 AND ANTIBOD?
                E LEVY DAVID/IN
L5            23 S E3
L6            23 S L5 NOT L4
                E REFAELI YOSEF/IN
L7             6 S E3
L8             0 S L7 NOT (L5 OR L1)
                E MONTAGNIER LUC/IN
L9            99 S E3
L10            9 S L9 AND (VPR OR VIRAL PROTEIN R)

=> s (HIV or human immunodeficiency virus)
         31488 HIV
        392203 HUMAN
         18172 IMMUNODEFICIENCY
         74782 VIRUS
         13017 HUMAN IMMUNODEFICIENCY VIRUS
                  (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L11      33171 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l11 and (Vpr or viral protein R)
          1091 VPR
         65975 VIRAL
        174530 PROTEIN
        977413 R
            32 VIRAL PROTEIN R
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L12          594 L11 AND (VPR OR VIRAL PROTEIN R)

=> s l12 and antibod?
        103710 ANTIBOD?
L13          548 L12 AND ANTIBOD?

=> s l13 and (antibod?/clm)
          31416 ANTIBOD?/CLM
L14          156 L13 AND (ANTIBOD?/CLM)

=> s l14 and (Vpr/clm or viral protein/clm)
            140 VPR/CLM
           9569 VIRAL/CLM
          50056 PROTEIN/CLM
            341 VIRAL PROTEIN/CLM
                  ((VIRAL(W)PROTEIN)/CLM)
L15           35 L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)

=> d his

     (FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
                  E WEINER DAVID/IN
L1             40 S E5
L2             49 S E3 OR E5
L3             26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4             26 S L3 AND ANTIBOD?
                  E LEVY DAVID/IN
L5             23 S E3
L6             23 S L5 NOT L4
                  E REFAELI YOSEF/IN
L7              6 S E3
L8              0 S L7 NOT (L5 OR L1)
                  E MONTAGNIER LUC/IN
L9             99 S E3
L10             9 S L9 AND (VPR OR VIRAL PROTEIN R)
L11         33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L12           594 S L11 AND (VPR OR VIRAL PROTEIN R)
L13           548 S L12 AND ANTIBOD?
L14           156 S L13 AND (ANTIBOD?/CLM)
L15            35 S L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)

=> s l15 not (l1 or l9)
L16           26 L15 NOT (L1 OR L9)

=> d l16,cbib,ab,clm,1-26

L16  ANSWER 1 OF 26  USPATFULL on STN
2004:144461 Compositions and methods for evaluating viral receptor/co-receptor
    usage and inhibitors of virus entry using recombinant virus assays.
    Petropoulos, Christos J., Half Moon Bay, CA, UNITED STATES
    Parkin, Neil T., Belmont, CA, UNITED STATES
    Whitcomb, Jeannette, San Mateo, CA, UNITED STATES
    Huang, Wei, Foster City, CA, UNITED STATES
    US 2004110125 A1 20040610
    APPLICATION: US 2002-164290  A1  20020604 (10)
    PRIORITY: US 2001-295871P  20010604 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     The invention provides a method for identifying whether a compound
       inhibits entry of a virus into a cell which comprises: (a) obtaining
       nucleic acid encoding a viral envelope protein from a patient infected
       by the virus; (b) co-transfecting into a first cell (i) the nucleic acid
       of step (a), and (ii) a viral expression vector which lacks a nucleic
```

acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the compound, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the compound indicates that the compound inhibits entry of the virus into the second cell.

CLM What is claimed is:

1. A method for identifying whether a compound inhibits entry of a virus into a cell which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the compound, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the compound indicates that the compound inhibits entry of the virus into the second cell.

2. The method of claim 1, wherein the indicator nucleic acid comprises an indicator gene.

3. The method of claim 2, wherein the indicator gene is a luciferase gene.

4. The method of claim 1, wherein the cell surface receptor is CD4.

5. The method of claim 1, wherein the cell surface receptor is a chemokine receptor.

6. The method of claim 1, wherein the cell surface receptor is CXCR4 or CCR5.

7. The method of claim 1, wherein the patient is infected with the **HIV**-1 virus.

8. The method of claim 1, wherein the nucleic acid of step (a) comprises DNA encoding gp120and gp41.

9. The method of claim 1, wherein the viral expression vector comprises **HIV** nucleic acid.

10. The method of claim 9, wherein the viral expression vector comprises an **HIV** gag-pol gene.

11. The method of claim 9, wherein the viral expression vector comprises DNA encoding vif, **vpr**, tat, rev, vpu, and nef.

12. The method of claim 1, wherein the first cell is a mammalian cell.

13. The method of claim 12, wherein the mammalian cell is a human cell.

14. The method of claim 13, wherein the human cell is a human embryonic

kidney cell.

15. The method of claim 14, wherein the human embryonic kidney cell is a 293 cell.

16. The method of claim 1, wherein the second cell is a human T cell.

17. The method of claim 1, wherein the second cell is a human T cell leukemia cell line.

18. The method of claim 1, wherein the second cell is a peripheral blood mononuclear cell.

19. The method of claim 1, wherein the second cell is an astroglioma cell.

20. The method of claim 19, wherein the astroglioma cell is a U87 cell.

21. The method of claim 1, wherein the second cell is a human osteosarcoma cell.

22. The method of claim 2, wherein the human osteosarcoma cell is an HT4 cell.

23. The method of claim 1, wherein the compound binds to the cell surface receptor.

24. The method of claim 1, wherein the compound is a ligand of the cell surface receptor.

25. The method of claim 23, wherein the compound comprises an **antibody**.

26. The method of claim 1, wherein the compound inhibits membrane fusion.

27. The method of claim 1, wherein the compound is a peptide, a peptidomimetic, an organic molecule, or a synthetic compound.

28. The method of claim 1, wherein the compound binds the viral envelope protein.

29. A method for making a composition which comprises admixing the compound identified by claim 1 with a carrier.

30. The method of claim 29, wherein the carrier is saline, polyethylene glycol, a buffer solution, a starch, or an organic solvent.

31. A method for identifying a cell surface receptor which is bound by a virus upon infection of a cell by the virus which comprises: (a) obtaining viral particles which comprise (i) a viral nucleic acid and (ii) an indicator nucleic acid which produces a detectable signal; (b) contacting a cell which expresses a cell surface receptor with the viral particles from step (a); and (c) measuring the amount of detectable signal produced within the cell, wherein production of the signal indicates the cell surface receptor expressed by the cell is bound by the virus, thereby identifying the cell surface receptor as being bound by the virus upon infection of the cell.

32. A method for identifying whether an **antibody** inhibits entry of a virus into a cell which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid

obtained from the patient; (b) contacting the viral particles produced in step (b) with a second cell in the presence of the **antibody**, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the **antibody** indicates that the **antibody** inhibits entry of the virus into the second cell.

33. A method for determining susceptibility of a virus to a compound which inhibits viral cell entry which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal: such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the compound, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the compound indicates that the virus is susceptible to the compound.

34. A method for determining resistance of a virus to a compound which inhibits viral entry into a cell which comprises: (a) determining susceptibility of a virus to a compound according to the method of claim 33, wherein a nucleic acid encoding a viral envelope protein is obtained from a patient at a first time; (b) determining susceptibility of the virus to the compound according to the method of claim 33, wherein the nucleic acid encoding the viral envelope protein is obtained from the patient at a later second time; and (c) comparing the susceptibilities determined in steps (a) and (b), wherein a decrease in susceptibility at the later second time indicates resistance of the virus to the compound.

35. A method for identifying a mutation in a virus that confers resistance to a compound that inhibits viral entry into a cell which comprises: (a) determining the nucleic acid sequence or the amino acid sequence of the virus prior to any treatment of the virus with the compound; (b) obtaining a virus resistant to the compound; (c) determining the nucleic acid sequence or the amino acid sequence of the resistant virus from step (b) and (d) comparing the nucleic acid sequence or the amino acid sequences of steps (a) and (c), respectively, so as to identify the mutation in the virus that confers resistance to the compound.

36. The method of claim 35, wherein the virus obtained in step (b) is the virus of step (a) grown in the presence of the compound until resistance is developed.

37. The method of claim 35, wherein the virus obtained in step (b) is isolated from a patient which has been undergoing treatment with the compound.

L16 ANSWER 2 OF 26 USPATFULL on STN
2004:133292 Method for analysis of the phenoptypic characteristics of the **human immunodeficiency virus** (**HIV**).
Clavel, Francois, Paris, FRANCE
Mammano, Fabrizio, Paris, FRANCE
Race, Esther, Angleterre, FRANCE
Dam, Elisabeth, Paris, FRANCE

Troulin, Virginie, Paris, FRANCE
BIOALLIANCE PHARMA INSERM (non-U.S. corporation)
US 2004101828 A1 20040527
APPLICATION: US 2003-435659 A1 20030512 (10)
PRIORITY: FR 2000-14495 20001009
FR 2001-3970 20010323
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for analyzing the phenotypic characteristics shown by certain virus strains, particularly human immunodeficiency viruses, involving the construction of a recombinant virus obtained by homologous recombination.

The present invention also relates a kit comprising the primers, vectors, cell hosts, products and reagents required to carry out PCR amplification, and the products and reagents used to detect a marker, for the implementation of the method according to the invention.

CLM What is claimed is:

1. A method for analysis of a phenotypic characteristic of **HIV** viruses that are present in a biological sample from a patient, whereby said phenotypic characteristic results from one or more mutations of the viral genome that can influence the viral infection, wherein said method comprises: a) extracting nucleic acids that are contained in said biological sample, b) amplifying by PCR a segment of the nucleic acids of stage (a), each with a pair of primers that frame a nucleic acid sequence of the viral genome that can carry at least one mutation, c) preparing a vector that comprises the parts of a genome of an **HIV** virus that are necessary for the viral replication except for the amplified segment in stage (b), d) transfecting a first cellular host with: The nucleic acids that are obtained in stage (b), The vector that is prepared in stage (c), to obtain a chimerical virus by homologous recombination, e) culturing said first cellular host under conditions that make it possible to produce viral particles during a single replication cycle, f) infecting said first cellular host with viral particles that are obtained in stage (e) from at least a second cellular host that can be infected by an **HIV** virus or an **HIV**-pseudotype virus, g) detecting, quantifying, or both, the marker that is expressed in stage (f) so as to demonstrate at least one characteristic of the **HIV** viruses that are present in the biological sample.

2. The method of claim 1, wherein the amplifying by PCR of stage (b) is carried out with a pair of primers that frame a nucleic acid sequence that comprises all or part of a viral genome region that is selected from among: gag, pol, protease, reverse transcriptase, RNAse H, integrase, vif, **vpr**, tat, rev, vpu, env, nef, cis-active sequences, LTR, dimerization sequences, splicing-regulating sequences or the Rev response element (RRE).

3. The method of claim 1, wherein the amplifying by PCR of stage (b) is carried out with a pair of primers that frame a nucleic acid sequence that codes for a portion of the gag protein of the **human immunodeficiency virus** and a nucleic acid sequence that codes for the protease that can carry at least one mutation in the gene that codes for the protease and wherein the vector of stage (c) is constructed from a genome of an **HIV** virus where all or part of the gene that codes for the protease is deleted.

4. The method of claim 1, wherein the amplifying of stage (b) that carries at least one mutation in the gene that codes for the protease is made with a pair of primers:

(SEQ ID No: 1)
Fit A-: (5' TCA CCT AGA ACT TTA AAT GC 3') and

(SEQ ID No: 2)
Pro A-: (5' GGC AAA TAC TGG AGT ATT GTA TG3' 3'),

followed by a second amplification with a pair of primers:

(SEQ ID No:3)
Fit B: (5' AGA ACT TTA AAT GCA TGG GT 3' ) and

(SEQ ID No:4)
Pro B-: (5' GGA GTA TTG TAT GGA TTT TCA GG 3') ,

to obtain a DNA segment of 1488 base pairs that extend between residues 1237 and 2725 inclusive, and wherein the vector of stage (c) is a retroviral vector that is deleted from the region of the pol reading frame that codes for the **HIV**-1 protease that extends from residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a unique restriction site MluI.

5. The method of claim 1, wherein the amplifying by PCR of stage (b) is carried out with a pair of primers that frame a nucleic acid sequence that can carry at least one mutation in the gene that codes for the reverse transcriptase, and the transfecting of stage (c) is carried out with a first vector that is constructed from a genome of an **HIV** virus where all or part of the gene that codes for the reverse transcriptase is deleted.

6. The method of claim 1, wherein the amplifying of stage (b) is carried out with a pair of primers:

(SEQ ID No: 5)
MJ3 (5' AGT AGG ACC TAC ACC TGT CA 3') and

(SEQ ID No: 6)
RT-EXT (5' TTC CCA ATG CAT ATT GTG AG 3'),

followed by a second amplification stage with a pair of primers: A35 (5' TTG GTT GCA TAA ATT TTC CCA TTA GTC CTA TT 3') (SEQ ID No: 7) and RT-IN (5' TTC CCA ATG CAT ATT GTG AG 3') (SEQ ID No: 8) to obtain a DNA segment of 1530 base pairs that extend beyond codon 93 of the region that codes for the protease and beyond codon 503 of the region that codes for the polymerase (POL), and wherein the vector of stage (c) is a retroviral vector that is deleted from the region of the pol reading frame that codes for the **HIV**-1 reverse transcriptase that extends from residues 2618 to 2872 inclusive and comprises a unique restriction site MluI.

7. The method of claim 1, wherein the amplifying by PCR of stage (b) is carried out with a pair of primers that frame a nucleic acid sequence that codes for a portion of the gag protein, for the protease and for a portion of the reverse transcriptase of the **human immunodeficiency virus** that can carry at least one mutation in the nucleic acid sequence that codes for the gag protein or for the protease or for the reverse transcriptase.

8. The method of claim 1, wherein the amplifying of stage (b) is carried out with the pair of primers: gag+1 (5' AGGGGCAAATGGTACATCA 3') (SEQ ID No: 31) and RT-EX (SEQ ID No 6), followed by a second amplification stage with a pair of primers: Fit B+ (SEQ ID No: 1) and RT-IN (SEQ ID No: 8) to obtain a DNA segment of 2825 base pairs that extend between residues 1237 and 4062 and wherein the transfecting of stage (c) is carried out with a retroviral vector that is deleted from a portion of the gag gene and regions within the pol reading frame that codes for the protease and a portion of the **HIV**-1 reverse transcriptase that extends from residues 1507 to 3870 inclusive, deleted in the envelope region and comprising a unique restriction site NruI.

9. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to an inhibitor compound of reverse transcriptase, wherein said inhibitor compound of reverse transcriptase is added or not added to the second cellular host, prior to the infection of the latter by viral particles that are obtained in stage (e), and wherein stage (g) comprises the comparison of the expression of the marker gene with and without an inhibitor compound of the reverse transcriptase.

10. The method of claim 1, wherein the amplifying by PCR of stage (b) is carried out with a pair of primers that frame a nucleic acid sequence that can carry at least one mutation in the gene that codes for integrase, and the vector of stage (c) is a retroviral vector that is deleted from all or part of the gene that codes for integrase.

11. The method of claim 1, wherein the amplifying of stage (b) is carried out with the pair of primers:

INT B+ - 5'GTTACTAATAGAGGAAGACAAA3' (SEQ ID No: 9)
and

INT B- 5'TTTTGGTGTTATTAATGCT3', (SEQ ID No: 10) .

followed by a second amplification stage, with the pair of primers:

INT V+
5' CACCCTAACTGACACAACAA3' and (SEQ ID No:11)

INT V-
5' AAGGCCTTTCTTATAGCAGA3', (SEQ ID No:12)

to obtain a DNA fragment of 1460 base pairs that extend from residues 3950 to 5410 inclusive, wherein the vector of stage (c) is a retroviral vector that is deleted from the entire region of the pol reading frame that codes for the **HIV**-1 integrase that extends from residues 4228 to 5093 inclusive and from the region that codes for the viral envelope between positions 6343 and 7611 inclusive.

12. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to an inhibitor compound of the integrase, comprising adding or not adding said inhibitor compound of the integrase during stage (e), before stage (f), and wherein stage (g) comprises the comparison of the expression of the marker gene with and without an inhibitor compound of the integrase.

13. The method of claim 1, wherein the amplifying by PCR of stage (b) is carried out with a pair of primers that frame a nucleic acid sequence that can carry at least one mutation in the gene that codes for the envelope protein, and wherein the vector of stage (c) is a retroviral vector that is constructed from a genome of an **HIV** virus where all or part of the gene that codes for the envelope protein is deleted.

14. The method of claim 1, wherein the vector of stage (c) is a retroviral vector that is deleted from the entire region that codes for the extracellular portion of sub-unit gp41 of the **HIV**-1 envelope that extends from residues 7745 to 8263 inclusive, from the region of the **HIV**-1 genome that constitutes the Rev response element (RRE).

15. The method of claim 1, wherein the amplifying of stage (b) is carried out with a pair of primers:

FIN-A:
5' TCAAATATTACAGGGCTGCT3' and (SEQ ID No: 13)

FIN-B:
5' TAGCTGAAGAGGCACAGG3' (SEQ ID No: 14)

followed by a second amplification stage, carried out with the pair of primers:

FIN-C:
5' CTATTAACAAGAGATGGTGG3'and (SEQ ID No: 15)

FIN-D:
5' TCCACCTTCTTCTTCGATT3', (SEQ ID No: 16)

to obtain a DNA segment of 965 base pairs that extend from residues 7553 to 8517 inclusive and wherein the vector of stage (c) is a retrovital vector that is deleted from the entire region that codes for the extracellular portion of sub-unit gp41 of the **HIV**-1 envelope that extends from residues 7745 to 8263 inclusive and comprises a unique restriction site Mull.

16. The method of claim 1, wherein the amplifying of stage (b) with a pair of primers that frame a nucleic acid sequence that can carry at least one mutation in the gene that codes for the envelope protein is made with a pair of primers:

FuA:
5' AAGCAATGTATGCCCCTCCCAT3'and (SEQ ID No: 23)

FuB:
5' GGTGGTAGCTGAAGAGGCACAGG3', (SEQ ID No: 24)

followed by a second amplification stage, carried out with the primer:

FuC':
5' ATATGAGGGACAATTGGAGAAGTGA3' (SEQ ID No: 25

and a mixture of the following primers:

FuD1:
5' TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No: 26)
and

FuD2:
5' TCTGTCTTGCTCTCCACCTTCTTCTT3', (SEQ ID No: 27)

to obtain a DNA segment of 805 base pairs that extend from residues 7635 to 8440 inclusive, and the vector of stage (c) is a retroviral vector that is deleted from the entire region that codes for the extracellular portion of sub-unit gp41 of the **HIV**-1 envelope that extends from residues 7745 to 8263 inclusive and comprises a unique restriction site Mull.

17. The method of claim 1, wherein the amplifying of stage (b) is carried out with a pair of primers:

NEU-A:
5' TAGAAAGAGCAGAAGACAGTGGCAATG3' (SEQ ID No: 17) and

FIN-B: 5' TAGCTGAAGAGGCACAGG3', (SEQ ID No: 14)
followed by a second amplification stage, with the pair of primers:

NEU-C:
5' GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18)
and

FIN-D:
5' TCCACCTTCTTCTTCGATT3', (SEQ ID No: 16)

to obtain a DNA fragment of between 2106 and 2320 base pairs that

wherein the vector of stage (c) is a retroviral vector that is deleted from the entire region that codes for the majority of sub-unit gp120 and the extracellular portion of gp41 of the **HIV**-1 envelope that extends from residues 6480 to 8263 inclusive and comprises a unique restriction site M1u1.

18. The method of claim 1, wherein the amplifying of stage (b) with a pair of primers that frame a nucleic acid sequence that can carry at least one mutation in the gene that codes for the envelope protein is made with a pair of primers:

NEU-A:
5' TAGAAAGAGCAGAAGACAGTGGCAATG3' (SEQ ID No: 17) and

FuB: 5' GGTGGTAGCTGAAGAGGCACAGG3', (SEQ ID No: 24)
followed by a second amplification stage, with the primers:

NEU-C:
5' GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18) and a
mixture of the primers

FuD1:
5' TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No: 26)
and

FuD2:
5' TCTGTCTTGCTCTCCACCTTCCTTCTT3', (SEQ ID No: 27)

to obtain a DNA fragment of 2118 base pairs that extend from residues 6322 to 8440 inclusive, and the vector of stage (c) is a retroviral vector that is deleted from the entire region that codes for the majority of sub-unit gp120 and the extracellular portion of the gp41 of the **HIV**-1 envelope that extends from residues 6480 to 8263 inclusive and comprises a unique restriction site Mu11.

19. The method of claim 1, wherein the amplifying of stage (b) with a pair of primers that frame a nucleic acid sequence that can carry at least one mutation in the gene that codes for the envelope protein is made with the pair of primers:

ED3:
5'TTAGGCATCTCCTATGGCAGGAAGAAGCGG3' (SEQ ID No: 28) and

(SEQ ID No: 29)
E01: 5' TCCAGTCCCCCCTTTTCTTTTAAAAA3',

followed by a second amplification stage, with the primers:

E10: 5'GTGGGTCACAGTCTATTATGGGGT3' (SEQ ID No: 30)
and

FuB: 5'GGTGGTAGCTGAAGAGGCACAGG3' (SEQ ID No: 24)

to obtain a DNA fragment of 2200 base pairs that extend from residues 6322 to 8522 inclusive, and the vector of stage (c) is a retroviral vector that is deleted from the entire region that codes for the majority of sub-unit gp120 and the extracellular portion of the gp41 of the **HIV**-1 envelope that extends from residues 6480 to 8263 inclusive and comprises a unique restriction site Mull.

20. The method of claim 1, wherein the amplifying of stage (b) is carried out with a pair of primers:

(SEQ ID No: 19)
E00: 5' TAGAAAGAGCAGAAGACAGTGGCAATGA3' and

(SEQ ID No: 20)
ES8B: 5' CACTTCTCCAATTGTCCCTCA3',

followed by a second amplification stage with the pair of primers:

(SEQ ID No: 21)
E20: 5' GGGCCACACATGCCTGTGTACCCACAG3' and

(SEQ ID No: 22)
E115: 5' AGAAAAATTCCCCTCCACAATTAA3',

to obtain a DNA segment of 938 base pairs that extend from residues 6426 to 7364 inclusive, and wherein the vector of stage (c) is a retroviral vector that is deleted from the region, coding for the domains that extend from loop VI to loop V3 of the **HIV**-1 envelope that extends from 6617 to 7250 inclusive and comprises a unique restriction site NheI.

21. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to a fusion-inhibitor compound that targets the gp41 protein of **HIV**-1, wherein said fusion-inhibitor compound is added during the cultivation of the cellular host that is obtained in stage (e), before stage (f) and wherein stage (g) comprises the comparison of the expression of the marker gene with and without a fusion inhibitor compound targeting the gp41 of **HIV**-1.

22. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to a compound that inhibits the entry of said **HIV** virus into a target cell, wherein said compound that inhibits entry is added to the cellular host that is obtained in stage (e) before the infection of stage (f) and wherein stage (g) comprises the comparison of the expression of the marker gene with and without a compound that inhibits entry.

23. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to the inhibiting action of **antibodies**, wherein said method is carried out without **antibodies** and with **antibodies** whereby said **antibodies** are present in stage (e) and wherein stage (g) comprises the comparison of the expression of the marker gene with and without **antibodies**.

24. The method of claim 1, wherein said method further comprises determining the tropism of an **HIV** virus for a cellular receptor, wherein the infection of stage (f) with the viral particles that are obtained in stage (e) is carried out on two separate cellular hosts, and stage (g) comprises the comparison of the expression of the marker gene by each of the two separate cellular hosts.

25. The method of claim 24, wherein one of the two cellular hosts infected in stage (f) expresses the receptor CCR5 and the other expresses the receptor CXCR4.

26. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to an inhibitor compound that targets the co receptors of **HIV**-1, wherein said inhibitor compound that targets the co-receptors of **HIV**-1 is added or not added during cultivation stage (e), wherein the infection of stage (f) is carried out on two separate cellular hosts and wherein stage (g) comprises the comparison of the expression of the marker gene by each of the two separate cellular hosts.

27. The method of claim 1, wherein said method further comprises analyzing the tropism of an **HIV** virus for a cellular receptacle, wherein the infection of stage (f) with the viral particles that are

obtained in stage (e), is carried out on two separate cellular hosts, and stage (g) comprises the comparison of the expression of the marker gene by each of the two separate cellular hosts.

28. The method of claim 1, wherein said method further comprises analyzing the susceptibility of an **HIV** virus to an inhibitor compound that targets the co-receptors of **HIV**-1, wherein said inhibitor compound that targets the co-receptors of **HIV**-1 is added during the cultivation of stage (d), wherein the infecting of stage (f) with the viral particles that are obtained in stage (e) is carried out on two separate cellular hosts and wherein stage (g) comprises the comparison of the expression of the marker gene by each of the two separate cellular hosts.

29. The method of claim 1, wherein said method further comprises determining the infectivity or the replicative capacity of an **HIV** virus, wherein stage (g) comprises the comparison of the expression of the marker gene by the second cellular host that is infected with the viral particles that are obtained by applying stages (a) to (f) to a biological sample of a patient, and the expression of the marker gene by the same second cellular host infected with reference viral particles that are obtained by applying stages (a) to (f) to a sample that contains a reference virus.

30. The method of claim 29, wherein the reference viral particles that are obtained from a reference virus are viral particles that are obtained by the application of stages (a) to (f) to a biological sample of the same patient at a previous stage of the therapeutic treatment or before the latter.

31. The method of claim 1, wherein said method further comprises determining the susceptibility of an **HIV** virus to hydroxyurea, wherein hydroxyurea is added or not added either during cultivation stage (e), or to the second cellular host, before the infection of the latter in stage (f) and wherein stage (g) comprises the comparison of the expression of the marker gene with and without hydroxyurea.

32. The method of claim 1, wherein the cultivating stage (e) is carried out during a period from 12 hours to 72 hours.

33. A kit for the implementation of a method according to claim 1, wherein said kit comprises: i. A pair of primers that frame a nucleic acid sequence of the viral genome that can carry at least one mutation, ii. a vector that comprises the parts of a genome of an **HIV** virus that are necessary to the viral replication except for the segment amplified with the primers that are defined by (i) and the gene that codes for the envelope protein, iii. a second vector that comprises a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

34. The kit of claim 33, wherein said kit comprises: i. The sequence primer pairs: SEQ ID No: 1 and SEQ ID No: 2 SEQ ID No: 3 and SEQ ID No: 4 ii. a retroviral vector that is deleted from the region of the pol reading frame that codes for the **HIV**-1 protease that extends from residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a unique restriction site M1u1, iii. a pseudotype virus with a gene that codes for an envelope virus, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the

expressed marker.

35. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: SEQ ID No: 5 and SEQ ID No: 7 SEQ ID No: 6 and SEQ ID No: 8 ii. a retroviral vector that is deleted from the region of the pol reading frame that codes for the **HIV**-1 reverse transcriptase that extends from residues 2618 to 2872 inclusive and that comprises a unique restriction site MluI, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

36. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: SEQ ID No: 9 and SEQ ID No: 10 SEQ ID No: 11 and SEQ ID No: 12 ii. a retroviral vector that is deleted from the entire region of the pol reading frame that codes for the **HIV**-1 integrase that extends from residues 4228 to 5093 inclusive and the region that codes for the viral envelope between positions 6343 and 7611 inclusive, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

37. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: SEQ ID No: 13 and SEQ ID No: 14 SEQ ID No: 15 and SEQ ID No: 16 ii. a retroviral vector that is deleted from the entire region that codes for the extracellular portion of sub-unit gp41 of the **HIV**-1 envelope that extends from residues 7745 to 8263 inclusive and comprises a unique restriction site Mu11, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a market gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

38. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: SEQ ID No: 23 and SEQ ID No: 24 SEQ ID No: 25 and SEQ ID No: 26 with SEQ ID No: 27 i. a retroviral vector that is deleted from the entire region that codes for the extracellular portion of sub-unit gp41 of the **HIV**-1 envelope that extends from residues 7745 to 8263 inclusive and comprises a unique restriction site Mu1, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

39. The kit of claim 33, wherein said kitcomprises: i. the sequence primer pairs: SEQ ID No: 17 and SEQ ID No: 14 SEQ ID No: 18 and SEQ ID No: 16 ii. a retroviral vector that is deleted from the entire region that codes for the majority of sub-unit gp120 and the extracellular portion of the gp41 of the **HIV**-1 envelope that extends from residues 6480 to 8263 inclusive and comprises a unique restriction site Mu11, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV**

virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only by viral particles, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

40. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: SEQ ID No: 17 and SEQ ID No: 24 SEQ ID No: 18 and SEQ ID No: 26 and SEQ ID No: 27 ii. a retroviral vector that is deleted from the entire region that codes for the majority of sub-unit gp120 and the extracellular portion of the gp41 of the **HIV**-1 envelope that extends from residues 6480 to 8263 inclusive and comprises a unique restriction site MulI, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only by viral particles, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

41. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: SEQ ID No: 19 and SEQ ID No: 20 SEQ ID No: 21 and SEQ ID No: 22 ii. a retroviral vector that is deleted from the region, coding for the domaims that extend from loop VI to loop V3 of the **HIV**-1 envelope that extends from 6617 to 7250 inclusive and that comprises a unique restriction site NheI, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

42. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: (SEQ ID No: 5) and (SEQ ID No: 6), (SEQ ID No: 7) and (SEQ ID No: 8) ii. a retroviral vector that is deleted from the region of the pol reading frame that codes for the **HIV**-1 reverse transcriptase that extends from residues 2618 to 2872 inclusive and comprises a unique restriction site MluI, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

43. The kit of claim 33, wherein said kit comprises: i. the sequence primer pairs: (SEQ ID No: 28) and (SEQ ID No: 29) and (SEQ ID No: 30) and (SEQ ID No: 24) ii. a retroviral vector that is deleted from the entire region that codes for the majority of sub-unit gp120 and the extracellular portion of the gp41 of the **HIV**-1 envelope that extends from residues 6480 to 8263 inclusive and comprises a unique restriction site MulI, iii. a virus that is pseudotyped by a gene that codes for an envelope protein, iv. a first cellular host that can be infected by an **HIV** virus, v. a second cellular host that can be infected by an **HIV** virus and that comprises a marker gene that can be activated only following viral infection, vi. the products and reagents that are necessary for carrying out the amplification by PCR, vii. the products and reagents that make it possible to detect the expressed marker.

44. The method of claim 1, wherein step (c) further comprises the parts of the genome of an **HIV** virus that are necessary for the viral replication except for the amplified segment in stage (b) and with the exception of the gene that codes for the envelope protein.

45. The method of claim 1, wherein step (d) further comprises transfecting a first cellular host with a second vector that comprises a gene that codes for an envelope protein if the envelope gene is deleted from the vector that is prepared in stage (c).

46. The method of claim 1, wherein the viral particles of step (f) further comprise a marker gene that can be activated only following viral infection.

47. The method of claim 9, wherein said inhibitor compound of reverse transcriptase is added or not added at different concentrations to the second cellular host.

48. The method of claim 12, wherein said inhibitor compound of the integrase is added or not added at different concentrations.

49. The method of claim 16, wherein said mixture of FuD1 and FuD2 is carried out in a ratio of between (10%:90%) and (90%:10%).

50. The method of claim 49, wherein said mixture is carried out in a ration of between (60%:40%) and (40%:60%).

51. The method of claim 18, wherein said mixture of FuD1 and FuD2 is carried out in a ratio that is between (10%:90%) and (90%:10%).

52. The method of claim 51, wherein said mixture is carrided out in a ratio that is between (60%:40%) and (40%:60%).

53. The method of claim 21, wherein said fusion-inhibitor compound is added at different concentrations during the cultivation of the cellular host.

54. The method of claim 22, wherein said compound that inhibits entry is added at different concentrations to the cellular host.

55. The method of claim 23, wherein said method is carried out without **antibodies** and with **antibodies** at different concentrations.

56. The method of claim 26, wherein said inhibitor compound that targets the co-receptors of **HIV**-1 is added or not added at different concentrations during cultivation stage (e).

57. The method of claim 28, wherein said inhibitor compound that targets the co-receptors of **HIV**-1 is added at different concentrations during the cultivation of stage (d).

58. The method of claim 31, wherein the hydroxyurea is added or not added at different concentrations.

59. The method of claim 32, wherein the cultivating stage (e) is carried out during a period from 24 hours to 48 hours.

L16 ANSWER 3 OF 26 USPATFULL on STN
2004:95552 Recombinant poxvirus for chimeric proteins of the **human immunodeficiency virus**.
Iglesias Perez, Enrique, Habana, CUBA
Vazquez Blomquist, Dania M, Ciudad Habana, CUBA
Duarte Cano, Carlos A, Ciudad Habana, CUBA
US 2004073008 A1 20040415
APPLICATION: US 2003-469256 A1 20030827 (10)
WO 2002-CU1 20020222
PRIORITY: CU 2001-572001 20010228
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ABS The invention relates to an HIV chimeric gene formed by the union of fragments of different genes of said virus, wherein said fragments contains epitopes for cytotoxic T cells (CTL) or **HIV**-1 auxiliary T cells, which are presented by a wide range of antigens of type Major Histocompatibility Complex (HLA-I). Recombinant poxviruses are obtained from said genes, which are useful for prophylactic and therapeutic vaccination against **HIV**/AIDS infections, are capable of generating a protective immune cell response in vaccinated laboratory animals and are recognized by the CTL lymphocytes of **HIV**/AIDS patients.

CLM What is claimed is:

1. A chimeric gene containing fragments from different **HIV**-1 genes, where those fragments encodes for cytotoxic T cells (CTL) epitopes rich regions, which are presented by a wide range of Major Histocompatiblity Complex (HLA-1) antigens, and can also contain T helper (Th) cells epitopes from **HIV** and at least one B cell epitope that is the target of a monoclonal **antibody**.

2. A gene as described in claim 1 which encodes for a chimeric poliprotein containing fragments from at least one **HIV** structural protein and one **HIV** non-structural protein.

3. A gene as described in claim 2 which encodes for a chimeric poliprotein containing fragments from **HIV**-1 proteins Reverse Transcriptase, P24 and Nef, Th epitopes from gp120, gp41 and **vpr** and a B cell epitope from gp120.

4. A gene as described in claim 3 which encodes for a chimeric poliprotein containing fragments 203-259 from Reverse Transcriptase, 219-307 from P24, and 45-147 from Nef., Th cell epitopes T1 and T2 from gp120, 580-594 from gp41 and 566-580 from **vpr** and B cell epitope from the V3 region MN strain recognized by Mab 2C4.

5. A gene as described in claim 4, which DNA sequence corresponds essentially with that of cr3 gene.

6. A chimeric protein which amino acid sequence corresponds essentially with the sequence of the protein CR3.

7. A recombinant virus for an heterologous gene, which contains fragments from different **HIV**-1 genes, where those fragments encodes for CTL epitopes rich regions, which are presented by a wide range of HLA-1 antigens, and can contain also **HIV**-1 T helper cell epitopes and at least one B cell epitope recognized by a Mab.

8. A recombinant virus as described in claim 7 where the heterologous gene encodes for a chimeric protein containing fragments from at least one **HIV** structural protein and one **HIV** non-structural protein.

9. A recombinant virus as described in claim 8 where the heterologous gene encodes for a chimeric protein containing fragments from **HIV**-1 proteins RT, P24 and Nef, Th epitopes from gp120, gp41 and **vpr** and a B cell epitope from gp120.

10. A recombinant virus as described in claim 9 where the heterologous gene encodes for a chimeric protein containing fragments 203-259 from RT; 219-307 from P24, and 45-147 from NEF and Th cell epitopes T1 and T2 from gp120, 580-594 from gp41 and 566-580 from **vpr** and B cell epitope from the V3 region MN strain recognized by Mab 2C4.

11. A recombinant virus as described in claim 10 where the DNA sequence of the heterologous gene corresponds essentially with cr3.

12. A virus as described in claims 7-11 where this virus is a poxvirus.

13. A virus as described in claims 7-12 where this virus is an Avipoxvirus

14. A virus as described in claims 7-13 where this virus is Fowl Pox Virus.

15. A virus as described in claims 7-14 where this virus is FPCR3.

16. A virus as described in claims 7-14 where this virus is FPSCR3gpt.

17. A vaccine formulation containing: A recombinant virus as described in claims 7-16. A pharmaceutical acceptable vehicle.

18. The use of a vaccine formulation described in claim 17 to induce an immune response against **HIV** in AIDS patients or uninfected persons.

19. A preventive or therapeutic combination composed of the vaccine formulation described in claim 17 and an immunopotentiator substance.

20. A preventive or therapeutic combination as described in claim 19 where immunopotentiator substance is a cytokine.

21. A preventive or therapeutic combination as described in claim 20 where such cytokine is IL2.

22. A plasmid verctor containing chimeric gene as described in claims 1-5 under the control of a mammalian cells promoter.

23. A vaccine formulation containing: A recombinant plasmid vector as described in claim 22 A pharmaceutical acceptable vehicle.

24. The use of a vaccine formulation described in claim 23 to induce an immune response against different proteins of **HIV** in AIDS patients or uninfected persons.

25. A preventive or therapeutic combination composed of the vaccine formulation described in claim 23 and an immunopotentiator substance.

26. A preventive or therapeutic combination as described in claim 25 where immunopotentiator substance is a cytokine.

27. A preventive or therapeutic combination as described in claim 26 where such cytokine is IL2.

L16 ANSWER 4 OF 26 USPATFULL on STN
2004:82307 Methods for stable transduction of cells with viral vectors.
Humeau, Laurent, Germantown, MD, UNITED STATES
Han, Wei, Montgomery Village, MD, UNITED STATES
Lu, Xiaobin, Germantown, MD, UNITED STATES
Slepushkin, Vladimir, Damascus, MD, UNITED STATES
Lesher, Mechelle, Columbia, MD, UNITED STATES
Davis, Brian, Gaithersburg, MD, UNITED STATES
Chang, Yung-Nien, Cockeysville, MD, UNITED STATES
Dropulic, Boro, Ellicott City, MD, UNITED STATES
US 2004062756 A1 20040401
APPLICATION: US 2003-664331 A1 20030916 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods, as well as compositions related thereto, for the efficient transduction of cells using viral vectors. The efficiency of transduction is increased by contacting the cell to be transduced with one or more molecules that bind the cell surface. Contact with a cell surface binding molecule may occur before, after, or simultaneously with contact between the viral vector and the cell. The transduced vectors may be constructed to express a gene of interest, permitting the transduced cells to be used as therapeutic and prophylactic agents.

What is claimed is:

1. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising contacting the surface of said cells with both a lentiviral vector and at least one molecule which binds said cell surface wherein said contacting occurs in vitro or ex vivo and wherein greater than about 90% of the cells are stably transduced after about 14 days.

2. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs before contacting the cells with at least one cell surface binding molecule.

3. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs simultaneously with contacting the cells with at least one cell surface binding molecule.

4. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs after contacting the cells with at least one cell surface binding molecule.

5. The method of claim 1 where said contacting with a lentiviral vector occurs more than once.

6. The method of claim 1 wherein said lentiviral vector is derived from **HIV**-1.

7. The method of claim 1 wherein said cell surface binding molecule is an **antibody**, a ligand or a cell surface molecule.

8. The method of claim 1 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, **vpr**, vpu, tat or rev genes.

9. The method of claim 8 wherein said sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, **vpr**, vpu, tat or rev genes.

10. The method of claim 1 wherein said lentiviral vector is derived from **HIV**-2.

11. The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

12. The method of claim 11 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

13. The method of claim 1 wherein said lentiviral vector is a chimeric vector comprising **HIV**-1 and **HIV**-2 sequences.

14. The method of claim 1 wherein said hematopoietic cell is a CD4 positive cell.

15. The method of claim 1 wherein said hematopoietic cell is a lymphocyte.

16. The method of claim 15 wherein said lymphocyte is a CD4 or CD8 positive cell.

17. The method of claim 1 wherein said hematopoietic cell is a CD34 positive cell.

18. The method of claim 17 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or **antibodies** that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

19. The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or **antibodies** that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

20. The method of claim 1 wherein the said cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

21. The method of claim 20 wherein said at least one cell surface binding molecule is selected from compositions comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or **antibodies** that are cell surface binding analogs of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

22. The method of claim 14 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 **antibodies** and fragments thereof, CD28 **antibodies** and fragments thereof, and combinations of said **antibodies** and fragments thereof.

23. The method of claim 22 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 **antibodies** immobilized on coated beads.

24. The method of claim 3 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

25. The method of claim 24 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

26. The method of claim 25 wherein said cytokine is interleukin-2.

27. The method of claim 24 wherein said culturing is for about seven days.

28. The method of claim 24 wherein said culturing is for about 14 days.

29. The method of claim 3 wherein said contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

30. The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

31. A method to introduce genetic material into a living subject comprising introduction of a cell transduced by the method of claim 1.

32. The method of claim 4 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

33. The method of claim 1 wherein said contacting occurs ex vivo.

L16 ANSWER 5 OF 26 USPATFULL on STN
2004:69992 Method for analysing **human immunodeficiency virus (HIV)** phenotypic characteristics.
Clavel, Francois, Paris, FRANCE
Race, Esther, Montrouge, FRANCE
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US 2004053219 A1 20040318
APPLICATION: US 2003-436458 A1 20030513 (10)
PRIORITY: FR 2000-14495 20001110

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for analysing the phenotypic characteristics shown by certain virus strains, particularly human immunodeficiency viruses, involving the construction of a recombinant virus obtained by homologous recombination.

The present invention also relates a kit comprising the primers, vectors, cell hosts, products and reagents required to carry out PCR amplification, and the products and reagents used to detect a marker, for the implementation of the method according to the invention.

CLM What is claimed is:

1. Method for analysing a phenotypic characteristic of **HIV** viruses present in a biological specimen from a patient, said phenotypic characteristic resulting from one or more mutations of the viral genome liable to influence the viral infection, characterised in that it comprises: a) the extraction of the nucleic acids contained in said biological specimen, b) at least one PCR amplification of a segment of the nucleic acids from step (a), each with a pair of primers bordering a nucleic acid sequence of the viral genome liable to comprise at least one mutation, c) the preparation of a vector comprising the parts of an **HIV** virus genome required for viral replication except for the segment amplified in step (b) and, if applicable, except for the gene coding for envelope protein, d) the transfection of a first cell host with: the nucleic acids contained in step (b), the vector prepared in step (c), if applicable, a second vector comprising a gene coding for an envelope protein if the envelope gene is deleted from the vector prepared in step (c), to obtain a chimeric virus by homologous recombination, e) the culture of said first cell host under conditions enabling the production of viral particles during a single replication cycle, f) the infection by the viral particles obtained in step (e) of at least one second cell host liable to be infected by an **HIV** virus or an **HIV** pseudotype virus and comprising, if applicable, a marker gene that can only be activated following viral infection, g) the detection and/or quantification of the marker expressed in step (f) in order to detect at least one characteristic of the **HIV** viruses present in the biological specimen.

2. Analytical method according to claim 1 characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence comprising all or part of a viral genome region selected from: gag, pol, protease, reverse transcriptase, RNAse H, integrase, vif, **vpr**, tat, rev, vpu, env, nef, cis-active sequences, LTR, dimerisation sequences, splicing regulating sequences or Rev response element (RRE).

3. Analytical method according to any of claims 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence coding for a part of the gag protein of the human immune deficiency virus and a nucleic acid sequence coding for protease, liable to comprise at least one mutation in the gene coding for protease and, in that the vector from step (c) is constructed from an **HIV** virus genome in which all or part of the gene coding for protease is deleted.

4. Analytical method according to claims 1 to 3 characterised in that the amplification in step (b) comprising at least one mutation in the gene coding for protease is performed with a pair of primers:

(SEQ ID No:1)
Fit A-: (5' TCA CCT AGA ACT TTA AAT GC 3') and

(SEQ ID No:2)
Pro A-: (5' GGC AAA TAC TGG AGT ATT GTA TG3'3',

followed by a second amplification with a pair of primers:

(SEQ ID No:3)
Fit B: (5' AGA ACT TTA AAT GCA TGG GT 3') and

(SEQ ID No:4)
Pro B-: (5' GGA GTA TTG TAT GGA TTT TCA GG 3'),

to obtain a DNA segment with 1460 base pairs, ranging from the residues 3950 and 5410 inclusive, and in that the vector from step (c) is a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 protease ranging from the residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a single MluI restriction site.

5. Analytical method according to claims 1 or 2 characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for reverse transcriptase, and the transfection in step (c) is carried out with a first vector constructed from an **HIV** virus genome in which all or part of the gene coding for reverse transcriptase is deleted.

6. Analytical method according to claim 1, 2 or 5, characterised in that the amplification in step (b) is performed with a pair of primers:

MJ3
(5' AGT AGG ACC TAC ACC TGT CA 3') (SEQ ID No:5)
and

RT-EXT
(5' TTC CCA ATG CAT ATT GTG AG 3'), (SEQ ID No:6)
followed by a second amplification step with a pair of primers:

A35 (5' TTG GTT GCA TAA ATT TTC CCA TTA GTC CTA TT 3') and
(SEQ ID No:7)

RT-IN (5' TTC CCA ATG CAT ATT GTG AG 3')
(SEQ ID No:8)

to obtain a DNA segment with 1530 base pairs ranging beyond codon 93 of the region coding for protease and beyond codon 503 of the region coding for polymerase (POL) and in that the vector from step (c) is a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 reverse transcriptase ranging from the residues 2618 to 2872 inclusive, and comprising a single MluI restriction site.

7. Analytical method according to claims 1, 2, 5 or 6 consisting of determining the susceptibility of an **HIV** virus to a reverse transcriptase inhibiting compound, characterised in that said reverse transcriptase inhibiting compound is added or not, possibly at different concentrations, to the second cell host, before the infection of said host by the viral particles obtained in step (e), and in that step (g) comprises the comparison of the expression of the marker gene with and without reverse transcriptase inhibiting compound.

8. Analytical method according to claims 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for integrase, and the vector in step (c) is a retroviral vector in which all or part of the gene coding for integrase is deleted.

9. Analytical method according to claim 1, 2 or 8, characterised in that the amplification in step (b) is performed with the pair of primers:

B+

INT 5'TTTTGGTGTTATTAATGCT3', (SEQ ID No:10)
B- followed by a second amplification step with the pair of primers:

INT V+ 5'CACCCTAACTGACACAACAA3' and (SEQ ID No:11)

INT V- 5'AAGGCCTTTCTTATAGCAGA3', (SEQ ID No:12)

to obtain a DNA segment with 1460 base pairs ranging from residues 3950 to 5410 inclusive and in that the vector from step (c) is a retroviral vector deleted from the entire region of the pol reading frame coding for **HIV**-1 integrase ranging from the residues 4228 to 5093 inclusive and the region coding for the viral envelope between the positions 6343 and 7611 inclusive.

10. Analytical method according to claims 1, 2, 8 or 9 consisting of determining the susceptibility of an **HIV** virus to an integrase inhibiting compound, characterised in that said integrase inhibiting compound is added, possibly at different concentrations, during step (e), before step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without integrase inhibiting compound.

11. Analytical method according to claims 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for envelope protein, and in that the vector from step (c) is a retroviral vector constructed from an **HIV** virus genome in which all or part of the gene coding for envelope protein is deleted.

12. Analytical method according to claims 1, 2 or 11 characterised in that the vector from step (c) is a retroviral vector deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, the region of the **HIV**-1 genome forming the Rev response element (RRE).

13. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: FIN-A: 5'TCAAATATTACAGGGCTGCT3' (SEQ ID No: 13) and FIN-B: 5'TAGCTGAAGAGGCACAGG3' (SEQ ID No: 14) followed by a second amplification step, performed with the pair of primers:

FIN-C: 5'CTATTAACAAGAGATGGTGG3' and (SEQ ID No:15)

FIN-D: 5'TCCACCTTCTTCTTCGATT3', (SEQ ID No:16)

to obtain a DNA segment with 965 base pairs ranging from the residues 7553 to 8517 inclusive and in that the vector in step (c) is a retroviral virus deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, and comprises a single MulI restriction site.

14. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers:

FuA: 5'AAGCAATGTATGCCCCTCCCAT3' and (SEQ ID No:23)

FuB: 5'GGTGGTAGCTGAAGAGGCACAGG3' (SEQ ID No:24)
followed by a second amplification step, performed with the primer: FuC: 5'ATATGAGGGACAATTGGAGAAGTGA3' (SEQ ID No: 25) and a mixture of the

following primers:

FuD1:
5'TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No:26)

FuD2:
5'TCTGTCTTGCTCTCCACCTTCTTCTT3', (SEQ ID No:27)

mixture being preferently carried out in a ratio comprised between (10%:90%) and (90%:10%) more preferently between (60%:40%) and (40%:60%), to obtain a DNA segment with 805 base pairs ranging from the residues 7635 to 8440 inclusive and the vector in step c is a retroviral virus deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, and comprises a single MulI restriction site.

15. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers:

NEU-A:
5'TAGAAAGAGCAGAAGACAGTGGCAATG3' and (SEQ ID No:17)

FIN-B:
5'TAGCTGAAGAGGCACAGG3' (SEQ ID No:14) second amplification step, performed with the pair of primers:

NEU-C: 5'GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No:18)
and

FIN-D: 5'TCCACCTTCTTCTTCGATT3', (SEQ ID No:16)

to obtain a DNA segment with 2320 base pairs ranging from the residues 6197 to 8517 inclusive and in that the vector in step (c) is a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 6480 to 8263 inclusive, and comprises a single MulI restriction site.

16. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers:

NEU-A:
5'TAGAAAGAGCAGAAGACAGTGGCAATG3' and (SEQ ID No:17)

FuB:
5'GGTGGTAGCTGAAGAGGCACAGG3', (SEQ ID No:24)
followed by a second amplification step, performed with the pair of primers: NEU-C: 5'GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18) and a mixture of the following primers

FuD1:
5'TCTGTCTCTCTCTCCACCTTCTTCTT3' and (SEQ ID No:26)

FuD2:
5'TCTGTCTTGCTCTCCACCTTCTTCTT3', (SEQ ID No:27)

mixture being preferently carried out in a ratio comprised between (10%:90%) and (90%:10%) more preferently between (60%:40%) and (40%:60%), to obtain a DNA segment with 2118 base pairs ranging from the residues 6322 to 8440 inclusive and the vector in step c is a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 6480 to 8263

inclusive, and comprises a single NdeI restriction site.

17. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers:

E00:
5'TAGAAAGAGCAGAAGACAGTGGCAATGA3' and (SEQ ID No:19)

ES8B:
5'CACTTCTCCAATTGTCCCTCA3', (SEQ ID No:22)
followed by a second amplification step, performed with the pair of primers:

E20:
5'GGGCCACACATGCCTGTGTACCCACAG3' and (SEQ ID No:21)

E115:
5'AGAAAAATTCCCCTCCACAATTAA3', (SEQ ID No:22)

to obtain a DNA segment with 938 base pairs ranging from the residues 6426 to 7364 inclusive and in that the vector in step (c) is a retroviral vector deleted from the region, coding for the domains ranging from the loop V1 to the loop V3 of the **HIV**-1 envelope ranging from 6617 to 7250 inclusive and comprises a single NheI restriction site.

18. Analytical method according to claims 1, 2, 11 to 17 consisting of determining the susceptibility of an **HIV** virus to a fusion inhibiting compound targeting **HIV**-1 gp41 protein, characterised in that said fusion inhibiting compound is added, possibly at different concentrations, during the culture of the cell host obtained in step (e), before step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without fusion inhibiting compound targeting **HIV**-1 gp41.

19. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to a compound inhibiting the entry of said **HIV** virus into a target cell, characterised in that said entry inhibiting compound is added, possibly at different concentrations, to the cell host obtained in step (e) before the infection in step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without entry inhibiting compound.

20. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to the inhibitory action of **antibodies**, characterised in that said method is carried out, firstly without **antibodies** and, secondly, with the **antibody**, possibly at different concentrations, said **antibody** being present in step (e), and in that step (g) comprises the comparison of the expression of the marker gene with and without **antibodies**.

21. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the tropism of an **HIV** virus for a cell receptor, characterised in that the infection in step (f) with the viral particles obtained in step (e) is performed on two separate cell hosts and step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

22. Analytical method according to claim 21 characterised in that one of two cell hosts infected in step (g) expresses the CCR5 receptor and the other expresses the CXCR4 receptor.

23. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to an inhibiting

compound targeting **HIV**-1 co-receptors, characterised in that said inhibiting compound targeting **HIV**-1 co-receptors is added or not, possibly at different concentrations, during the culture step (e), in that the infection in step (f) is performed on two separate cell hosts and in that step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

24. Analytical method according to claims 1, 2, 11, 12 or 17 consisting of analysing the tropism of an **HIV** virus for a cell receptor, characterised in that the infection in step (f) with the viral particles obtained in step (e) is performed on two separate cell hosts and step (g) comprises a comparison of the expression of the marker gene by each of the two separate cell hosts.

25. Analytical method according to claims 1, 2, 11, 12 or 17 consisting of analysing the susceptibility of an **HIV** virus to an inhibiting compound targeting **HIV**-1 co-receptors, characterised in that said inhibiting compound targeting **HIV**-1 co-receptors is added, possibly at different concentrations, during the culture in step (d), in that the infection in step (i) with the viral particles in step (e) is performed on two separate cell hosts and in that step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

26. Analytical method according to any of claims 1 to 17 consisting of determining the infectivity or replicative capacity of an **HIV** virus characterised in that step (g) comprises the comparison of the expression of the marker gene by the second cell host infected with the viral particles obtained by applying steps (a) to (f) to a biological specimen from a patient, and the expression of the marker gene by the same second cell host infected with the reference viral particles obtained by applying steps (a) to (f) to a specimen containing a reference virus.

27. Analytical method according to claim 26 characterised in that the reference viral particles from a reference virus are viral particles obtained by applying steps (a) to (f) to a biological specimen from the same patient at an earlier stage or treatment or before said treatment.

28. Analytical method according to claims 1 to 17 consisting of determining the susceptibility of an **HIV** virus to hydroxyurea, characterised in that hydroxyurea is added or not, possibly at different concentrations, either during the culture step (e), or to the second cell host, before the infection of said host in step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without hydroxyurea.

29. Analytical method according to any of claims 1 to 28 characterised in that the culture step (e) is performed during a period ranging from 12 hours to 72 hours, preferentially from 24 hours to 48 hours.

30. A kit for implementing the method according to any of claims 1 to 29 characterised in that it comprises: i) a pair of primers bordering a nucleic acid sequence of the viral genome liable to comprise at least one mutation, ii) a vector comprising the parts of an **HIV** virus genome required for viral replication except for the segment amplified with the primers defined in (i) and the gene coding for the envelope protein, iii) a second vector comprising a gene coding for envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

31. A kit according to claim 30, characterised in that it comprises: i)

the sequence primer pairs:

SEQ ID No:1 and SEQ ID No:2

SEQ ID No:3 and SEQ ID No:4

ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 protease ranging from the residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a single MluI restriction site, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

32. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs:

SEQ ID No:5 and SEQ ID No:7

SEQ ID No:6 and SEQ ID No:8

ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 reverse transcriptase ranging from the residues 2618 to 2872 inclusive, and comprising a single MluI restriction site, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

33. A kit according to claim 30 characterised in that it comprises: i) the sequence primer pairs:

SEQ ID No:9 and SEQ ID No:10

SEQ ID No:11 and SEQ ID No:12

ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 integrase ranging from the residues 4228 to 5093 inclusive and the region coding for the viral envelope between the positions 6343 and 7611 inclusive, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and. reagents used to detect the expressed marker.

34. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs:

SEQ ID No:13 and SEQ ID No:14

SEQ ID No:15 and SEQ ID No:16

ii) a retroviral vector deleted from the entire region coding for the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 7745 to 8263 inclusive, and comprising a single MluI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out

PCR amplification, vii) the products and reagents used to detect the expressed marker.

35. A kit according to claim 30, characterised in that it comprises: i) the sequence primers: (SEQ ID No: 23) and (SEQ ID No: 24) (SEQ ID No: 25) and the mixture of primers (SEQ ID No: 26) and (SEQ ID No: 27), ii) a retroviral vector deleted from the entire region coding for the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 7745 to 8263 inclusive, and comprising a single MluI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

36. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs:

SEQ ID No:17 and SEQ ID No:14

SEQ ID No:18 and SEQ ID No:16

ii) a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 6480 to 8263 inclusive, and comprising a single MulI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated by viral particles, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

37. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs:

SEQ ID No:17 and SEQ ID No:24

SEQ ID No:18 and SEQ ID No:26 and SEQ ID No:27

ii) a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 6480 to 8263 inclusive, and comprising a single MulI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated by viral particles, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker

38. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs:

SEQ ID No:19 and SEQ ID No:20

SEQ ID No:21 and SEQ ID No:22

ii) a retroviral vector deleted from the region, coding for the domains ranging from the loop V1 to the loop V3 of the **HIV**-1 envelope, ranging from 6617 to 7250 inclusive, and comprising a single NheI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

L16 ANSWER 6 OF 26 USPATFULL on STN
2004:13387 Methods for inhibiting lentivirus replication.
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US 2004009909 A1 20040115
APPLICATION: US 2002-285263 A1 20021030 (10)
PRIORITY: US 2001-350168P 20011102 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides screening methods for identifying a compound that induces loss of the lentiviral protein **Vpr**; screening methods for identifying compounds that inhibit the peptidyl-prolyl cis/trans isomerase (PPIase) activity of a protein that catalyzes cis-trans isomerization of cis-peptidylprolyl bonds in **Vpr**; and compounds identified by the screening methods. The compounds are useful for treating a lentiviral infection. The present invention further provides methods of inducing loss of the lentiviral protein **Vpr**; methods of inhibiting lentivirus viral replication; and methods of treating a lentivirus infection in an individual. The methods generally involve administering to an individual infected with the lentivirus an effective amount of a compound that induces **Vpr** loss and/or that inhibits PPIase activity of a protein that catalyzes cis-trans isomerization of cis-peptidylprolyl bonds in **Vpr**.

CLM What is claimed is:

1. A method of identifying an agent that induces **Vpr** loss in a lentivirus-infected cell, the method comprising: a) contacting a cell that produces a **Vpr** protein with a test agent; and b) determining the effect, if any, of the test agent on the level of **Vpr** in the cell.

2. The method of claim 1, wherein **Vpr** protein is a fusion protein comprising **Vpr** and a fusion partner that provides a detectable signal.

3. The method of claim 2, wherein the fusion partner is selected from a fluorescent protein, an enzyme, and an immunological tag.

4. The method of claim 1, wherein said determining step is an immunological assay using an **antibody** specific for **Vpr**.

5. The method of claim 1, further comprising determining the effect, if any, of the agent on a peptidyl-prolyl cis/trans isomerase (PPIase) activity of a PPIase protein that catalyzes cis-trans isomerization of cis-peptidylprolyl bonds in **Vpr**.

6. The method of claim 6, wherein said determining comprises detecting PPIase activity using as a substrate a compound of the formula Xaa-Ala-Xaa-Pro-Phe-X, where Xaa is any amino acid, and wherein X is a moiety that provides a detectable signal.

7. The method of claim 6, wherein the compound is Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide.

8. The method of claim 1, further comprising determining the effect, if any, of the agent on **Vpr**-induced cell cycle arrest.

9. The method of claim 8, wherein said determining step comprises determining the DNA complement of the cell.

10. The method of claim 9, wherein said determining step comprises staining the cell with a DNA binding dye.

11. The method of claim 10, wherein the DNA binding dye is selected from propidium iodide, and a Hoechst dye.

12. An agent identified by a method according to claim 1, wherein said agent induces **Vpr** loss in a lentivirus-infected cell.

13. The agent of claim 12, wherein said agent inhibits a peptidyl-prolyl cis/trans isomerase activity of a PPIase protein that catalyzes cis-trans isomerization of cis-peptidylprolyl bonds in **Vpr**.

14. The agent of claim 12, wherein said agent inhibits **Vpr**-induced cell cycle arrest.

15. The agent of claim 12, wherein said agent does not inhibit binding of **HIV**-Gag to cyclophilinA.

16. A pharmaceutical composition comprising: a) an agent according to claim 12; and b) a pharmaceutically acceptable excipient.

17. The pharmaceutical composition according to claim 16, further comprising an effective amount of an anti-**HIV** therapeutic agent.

18. A method of inducing **Vpr** loss in a cell infected with a lentivirus, the method comprising: contacting the cell with an agent according to claim 12.

19. A method of treating a lentivirus infection in an individual, the method comprising: administering to the individual an effective amount of a composition according to claim 16, wherein a reduction in the level of **Vpr** in the cell treats a lentivirus infection.

20. The method of claim 19, wherein viral load is reduced.

L16 ANSWER 7 OF 26 USPATFULL on STN
2003:312671 **HIV** vaccine and method of use.
Narayan, Opendra, Lenexa, KS, UNITED STATES
US 2003220276 A1 20031127
APPLICATION: US 2002-279992 A1 20021024 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a vaccine for immunization against **HIV**. The vaccine has DNA sequences encoding a plurality of viral proteins, including NEF, VPU and reverse transcriptase. The vaccine is rendered nonpathogenic by the disruption of the gene(s) encoding for at least one of these proteins.

CLM What is claimed is:

1. A vaccine for immunization against **HIV** comprising an isolated DNA molecule having a sequence encoding a plurality of viral proteins capable of stimulating an immune response against **HIV**, wherein the combination of viral proteins is rendered nonpathogenic by altering the DNA molecule such that it is unable to encode at least one functional protein selected from the group consisting of Nef, Vpu and reverse transcriptase.

2. A vaccine for immunization against **HIV** comprising an isolated DNA molecule having a sequence encoding a plurality of viral proteins capable of stimulating an immune response against **HIV**, wherein the combination of viral proteins is rendered nonpathogenic by altering the DNA molecule such that it is unable to encode a functional reverse transcriptase protein.

3. A vaccine for immunization against **HIV** comprising an isolated DNA molecule having a sequence encoding a plurality of viral proteins capable of stimulating an immune response against **HIV**, wherein the

combination of viral proteins is rendered nonpathogenic by altering the DNA molecule such that it is unable to encode a functional Nef protein.

4. A vaccine for immunization against **HIV** comprising an isolated DNA molecule having a sequence encoding a plurality of viral proteins capable of stimulating an immune response against **HIV**, wherein the combination of viral proteins is rendered nonpathogenic by altering the DNA molecule such that it is unable to encode a functional Vpu protein.

5. The vaccine of claim 2 wherein said DNA molecule is altered by at least a partial deletion of a reverse transcriptase gene.

6. The vaccine of claim 2 wherein the altered DNA molecule has a nonfunctional reverse transcriptase gene having the same number of nucleotides as a functional, unaltered reverse transcriptase gene.

7. The vaccine of claim 2 wherein said DNA further comprises a 3' LTR sequence and said DNA molecule has been further altered by at least a partial deletion of the 3' LTR sequence.

8. The vaccine of claim 3 wherein said DNA molecule is altered by at least a partial deletion of a nef gene.

9. The vaccine of claim 3 wherein the altered DNA molecule has a nonfunctional nef gene having the same number of nucleotides as a functional, unaltered nef gene.

10. The vaccine of claim 3 wherein said DNA further comprises a 3' LTR sequence and said DNA molecule has been further altered by at least a partial deletion of the 3' LTR sequence.

11. The vaccine of claim 4 wherein said DNA molecule is altered by at least a partial deletion of a vpu gene.

12. The vaccine of claim 4 wherein said DNA further comprises a 3' LTR sequence and said DNA molecule has been further altered by at least a partial deletion of the 3' LTR sequence.

13. The vaccine of claim 1 wherein said DNA further comprises a 3' LTR sequence and said DNA molecule has been further altered by at least a partial deletion of the 3' LTR sequence.

14. The vaccine of claim 1, wherein the DNA molecule is derived from an **HIV** virus having a protein capable of downregulating CD4 levels in vivo, and the combination of viral proteins is rendered nonpathogenic by disrupting the ability of said DNA molecule to encode for the protein capable of downregulating CD4 levels in vivo.

15. The vaccine of claim 1, wherein the DNA molecule is derived from an **HIV** virus having a protein essential to the ability of the **HIV** virus to induce disease, and the combination of viral proteins is rendered nonpathogenic by disrupting the ability of the DNA molecule to encode for the protein essential to the ability of the **HIV** virus to induce disease.

16. The vaccine of any one of claims 1 through 15 further comprising a pharmaceutically acceptable carrier.

17. The vaccine of any one of claims 1 through 15 further comprising a natural **HIV** promoter sequence.

18. The vaccine of any one of claims 1 through 15 further comprising a CMV promoter sequence.

19. A DNA immunogenic composition derived from a viral genome coding for at least one protein capable of providing an immune response against

repeat, wherein the ability of the DNA immunogenic composition to integrate into a host genome has been destroyed by disruption of the 3' long-terminal repeat.

20. A DNA immunogenic composition comprising the nucleotide sequence of SEQ ID NO:1.

21. A DNA immunogenic composition comprising the nucleotide sequence of SEQ ID NO:2.

22. A DNA immunogenic composition comprising the nucleotide sequence of SEQ ID NO:3.

23. A DNA immunogenic composition comprising the nucleotide sequence of SEQ ID NO:5.

24. A DNA immunogenic composition comprising the nucleotide sequence of SEQ ID NO:6.

25. The DNA immunogenic composition of any one of claims 20 through 24 further comprising a suitable vector.

26. The DNA immunogenic composition of any one of claims 20 through 24 further comprising a vector having the nucleotide sequence of SED ID NO:4.

27. A DNA immunogenic composition comprising a nucleotide sequence comprising: (a) the 5' LTR of SIV; (b) the gag gene of SIV; (c) the pro gene of SIV; (d) the int gene of SIV; (e) the vif gene of SIV; (f) the **vpr** gene of SIV; (g) the vpx gene of SIV; (h) the rt gene of SIV wherein the rt gene of SIV has been disrupted; (i) the env gene of **HIV**; (j) the vpu gene of **HIV**; (k) the nef gene of SIV; and (l) the 3' long-terminal repeat of SIV.

28. A DNA immunogenic composition according to claim 27 wherein said vpu gene has been disrupted.

29. A DNA immunogenic composition according to claim 27 wherein said nef gene has been disrupted.

30. A DNA immunogenic composition according to claim 27 wherein said 3' long terminal repeat has been disrupted.

31. A DNA immunogenic composition according to claim 27 wherein said vpu and nef genes have been disrupted.

32. A DNA immunogenic composition according to claim 27 wherein said vpu and nef genes have been disrupted and further wherein said 3'0 long terminal repeat has been disrupted.

33. A DNA immunogenic composition comprising a nucleotide sequence comprising: (a) the 5' LTR of SIV; (b) the gag gene of SIV; (c) the pro gene of SIV; (d) the int gene of SIV; (e) the vif gene of SIV; (f) the **vpr** gene of SIV; (g) the vpx gene of SIV; (h) the rt gene of SIV wherein the rt gene of SIV has been disrupted; (i) the env gene of **HIV**; (j) the vpu gene of **HIV**; (k) the nef gene of SIV; and (l) an SV 40 polyadenylation sequence.

34. A DNA immunogenic composition according to claim 33 wherein said vpu gene has been disrupted.

35. A DNA immunogenic composition according to claim 33 wherein said nef gene has been disrupted.

36. A DNA immunogenic composition according to claim 33 wherein said vpu

and nef genes have been disrupted.

37. A DNA immunogenic composition comprising a nucleotide sequence comprising: (a) the 5'0 LTR of SIV; (b) the gag gene of **HIV**; (c) the pro gene of **HIV**; (d) the int gene of SIV; (e) the vif gene of SIV; (f) the **vpr** gene of SIV; (g) the vpx gene of SIV; (h) the rt gene of SIV wherein the rt gene of SIV has been disrupted; (i) the env gene of **HIV**; (j) the vpu gene of **HIV**; (k) the nef gene of SIV; and (l) an SV 40 polyadenylation sequence.

38. A DNA immunogenic composition according to claim 37 wherein said vpu gene has been disrupted.

39. A DNA immunogenic composition according to claim 37 wherein said nef gene has been disrupted.

40. A DNA immunogenic composition according to claim 37 wherein said vpu and nef genes have been disrupted.

41. A method of providing vaccination against **HIV** comprising administering to a recipient the DNA composition of any one claims 1 through 15, 19 through 24, or 27 through 40.

42. A recombinant virus wherein the DNA of said recombinant virus comprises SIV LTR, gag, pol and nef genes and **HIV**-1 env, tat, and rev genes, and a nonfunctional vpu gene from **HIV**-1, wherein the vpu gene is rendered nonfunctional by at least a partial deletion of the vpu gene and further wherein said nonfunctional vpu gene does not have the same number of nucleotides as a functional **HIV**-1 vpu gene.

43. The recombinant virus of claim 42 wherein said nef gene is rendered nonfunctional by at least a partial deletion of said nef gene.

44. A DNA construct comprising SIV LTR, gag, pol and nef genes and **HIV**-1 env, tat, and rev genes, and a nonfunctional vpu gene from **HIV**-1, wherein the vpu gene is rendered nonfunctional by at least a partial deletion of the vpu gene and further wherein said nonfunctional vpu gene does not have the same number of nucleotides as a functional **HIV**-1 vpu gene.

45. The DNA construct of claim 44 wherein said nef gene is rendered nonfunctional by at least a partial deletion of said nef gene.

46. An **HIV**-1/**HIV**-2 Chimeric virus wherein the DNA of the Chimeric virus comprises **HIV**-2 LTR, gag, pol, and nef genes and **HIV**-1 env, tat and rev genes and, optionally, an **HIV**-1 vpu gene, wherein said vpu gene if present is rendered nonfunctional.

47. The Chimeric virus of claim 46, wherein the vpu gene if present has been rendered nonfunctional by at least a partial deletion of said vpu gene.

48. A method for the creation of an effective vaccine for conveying immunity to **HIV**-1 virus comprising manipulating the **HIV**-1 virus to impede its ability to effectively replicate and/or otherwise accumulate in the infected/inoculated host.

49. A method as in claim 48 wherein the manipulation is the interference with the activity of the vpu gene or gene product of the virus.

50. A method for the treatment of currently infected **HIV**-1 positive patients comprising administering agents that will interfere with the **HIV**-1 vpu gene or gene products wherein such agents can be chemical, **antibody**-based, or other form of bioavtive molecule.

51. A method for the treatment of currently infected **HIV**-1 positive

patients comprising administering agents that will interfere with the **HIV**-1 reverse transcriptase or gene products wherein such agents can be chemical, **antibody**-based, or other form of bioavtive molecule.

L16 ANSWER 8 OF 26 USPATFULL on STN
2003:294279 Method for analysing immunodeficiency virus (**HIV**) phenotypic characteristics.
Clavel, Francois, Paris, FRANCE
Race, Esther, Montrouge, FRANCE
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Bioalliance Pharma (S.A) (non-U.S. corporation)
US 2003207294 A1 20031106
APPLICATION: US 2002-263655 A1 20021004 (10)
PRIORITY: FR 2000-14495 20001110
FR 2001-3970 20010323
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for analysing the phenotypic characteristics shown by certain virus strains, particularly human immunodeficiency viruses, involving the construction of a recombinant virus obtained by homologous recombination.

The present invention also relates a kit comprising the primers, vectors, cell hosts, products and reagents required to carry out PCR amplification, and the products and reagents used to detect a market, for the implementation of the method according to the invention.
CLM What is claimed is:
1. Method for analysing a phenotypic characteristic of **HIV** viruses present in a biological specimen from a patient, said phenotypic characteristic resulting from one or more mutations of the viral genome liable to influence the viral infection, characterised in that it comprises: a) the extraction of the nucleic acids contained in said biological specimen, b) at least one PCR amplification of a segment of the nucleic acids from step (a), each with a pair of primers bordering a nucleic acid sequence of the viral genome liable to comprise at least one mutation, c) the preparation of a vector comprising the parts of an **HIV** virus genome required for viral replication except for the segment amplified in step (b) and, if applicable, except for the gene coding for envelope protein. d) the transfection of a first cell host with: the nucleic acids contained in step (b), the vector prepared in step (c), if applicable, a second vector comprising a gene coding for an envelope protein if the envelope gene is deleted from the vector prepared in step (c), to obtain a chimeric virus by homologous recombination, e) the culture of said first cell host under conditions enabling the production of viral particles during a single replication cycle, f) the infection by the viral particles obtained in step (e) of at least one second cell host liable to be infected by an **HIV** virus or an **HIV** pseudotype virus and comprising, if applicable, a marker gene that can only be activated following viral infection, g) the detection and/or quantification of the marker expressed in step (f) in order to detect at least one characteristic of the **HIV** viruses present in the biological specimen.

2. Analytical method according to claim 1 characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence comprising all or part of a viral genome region selected from: gag, pol, protease, reverse transcriptase, RNAse H, integrase, vif, **vpr**, tat, rcv, vpu, env, nef, cis-active sequences, LTR, dimerisation sequences, splicing regulating sequences or Rev response element (RRE).

3. Analytical method according to any of claims 1 or 2, characterised in

that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence coding for a part of the gag protein of the human immune deficiency virus and a nucleic acid sequence coding for protease, liable to comprise at least one mutation in the gene coding for protease and, in that the vector from step (c) is constructed from an **HIV** virus genome in which all or part of the gene coding for protease is deleted.

4. Analytical method according to claims 1 to 3 characterised in that the amplification in step (b) comprising at least one mutation in the gene coding for protease is performed with a pair of primers: Fit A-: (5' TCA CCT AGA ACT TTA AAT GC 3') (SEQ ID No: 1) and Pro A-: (5' GGC AAA TAC TGG AGT ATT GTA TG3'3' (SEQ ID No: 2), followed by a second amplification with a pair of primers: Fit B: (5' AGA ACT TTA AAT GCA TGG GT 3') (SEQ ID No: 3) and Pro B-: (5' GGA GTA TTG TAT GGA TTT TCA GG 3') (SEQ ID No: 4). to obtain a DNA segment with 1460 base pairs, ranging from the residues 3950 and 5410 inclusive, and in that the vector form step (c) is a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 protease ranging from the residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a single MluI restriction site.

5. Analytical method according to claims 1 or 2 characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for reverse transcriptase, and the transfection in step (c) is carried out with a first vector constructed from an **HIV** virus genome in which all or part of the gene coding for reverse transcriptase is deleted.

6. Analytical method according to claim 1, 2 or 5, characterised in that the amplification in step (b) is performed with a pair of primers: MJ3 (5' AGT AGG ACC TAC AC TGT CA 3') (SEQ ID No: 5) and RT-EXT (5' TTC CCA ATG CAT ATT GTG AG 3') (SEQ ID No: 6), followed by a second amplification step with a pair of primers: A35 (5' TTG GTT GCA TAA ATT TTC CCA TTA GTC CTA TT 3') (SEQ ID No: 7) and RT-IN (5' TTC CCA ATG CAT ATT GTG AG 3') (SEQ ID No: 8) to obtain a DNA segment with 1530 base pairs ranging beyond codon 93 of the region coding for protease and beyond codon 503 of the region coding for polymerase (POL) and in that the vector from step (c) is a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 reverse transcriptase ranging from the residues 2618 to 2872 inclusive, and comprising a single MluI restriction site.

7. Analytical method according to claims 1, 2, 5 or 6 consisting of determining the susceptibility of an **HIV** virus to a reverse transcriptase inhibiting compound, characterised in that said reverse transcriptase inhibiting compound is added or not, possibly at different concentrations, to the second cell host, before the infection of said host by the viral particles obtained in step (e), and in that step (g) comprises the comparison of the expression of the marker gene with and without reverse transcriptase inhibiting compound.

8. Analytical method according to claims 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for integrase, and the vector in step (c) is a retroviral vector in which all or part of the gene coding for integrase is deleted.

9. Analytical method according to claim 1, 2 or 8, characterised in that the amplification in step (b) is performed with the pair of primers: INT B+-5'GTTACTAATAGAGGAAGACAAAC3'(SEQ ID No:9) and INT B-5'TTTTGGTGTTATTAATGCT3' (SEQ ID No: 10), followed by a second amplification step with the pair of primers: INT V+ 5'CACCCTAACTGACACAACAA3' (SEQ ID No: 11) and INT V-

1460 base pairs ranging from residues 3950 to 5410 inclusive and in that the vector from step (c) is a retroviral vector deleted from the entire region of the pol reading frame coding for **HIV**-1 integrase ranging from the residues 4228 to 5093 inclusive and the region coding for the viral envelope between the positions 6343 and 7611 inclusive.

10. Analytical method according to claims 1, 2, 8 or 9 consisting of determining the susceptibility of an **HIV** virus to an integrase inhibiting compound, characterised in that said integrase inhibiting compound is added, possibly at different concentrations, during step (e), before step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without integrase inhibiting compound.

11. Analytical method according to claims 1 or 2, characteristed in that the PCR amplificaiton in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for envelope protein, and in that the vector from step (c) is a retroviral vector constructed from an **HIV** virus genome in which all or part of the gene coding for envelope protein is deleted.

12. Analytical method according to claims 1, 2 or 11 characterised in that the vector from step (c) is a retroviral vector deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, the region of the **HIV**-1 genome forming the Rev response element (RRE).

13. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: FIN-A: 5'TCAAATATTACAGGGCTGCT3' (SEQ ID No: 13) and FIN-B: 5'TAGCTGAAGAGGCACAGG3' (SEQ ID No: 14) followed by a second amplification step, performed with the pair of primers: FIN-C: 5'CTATTAACAAGAGATGGTGG3' (SEQ ID No: 15) and FIN-D: 5'TCCACCTTCTTCTTCGATT3' (SEQ ID No: 16), to obtain a DNA segment with 965 base pairs ranging from the residues 7553 to 8517 inclusive and in that the vector in step (c) is retroviral virus deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, and comprises a single MulI restriction site.

14. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: FuA: 5'AAGCAATGTATGCCCCTCCCAT3' (SEQ ID No: 23) and FuB: 5'GGTGGTAGCTGAAGAGGCACAGG3' (SEQ ID No: 24) followed by a second amplification step, performed with the primer: FuC: 5'ATATGAGGGACAATTGGAGAAGTGA3' (SEQ ID No: 25) and a mixture of the following primers: FuD1: 5'TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No: 26) FuD2: 5'TCTGTCTTGCTCTCCACCTTCTTCTT3' (SEQ ID No: 27), said mixture being preferently carried out in a ratio comprised between (10%:90%) and (90%:10%) more preferently between (60%:40%) and (40%:60%), to obtain a DNA segment with 805 base pairs ranging from the residues 7635 to 8440 inclusive and the vector in step c is a retroviral virus deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, and comoprises a single MulI restriction site.

15. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: NEU-A: 5'TAGAAAGAGCAGAAGACAGTGGCAATG3' (SEQ ID No: 17) and FIN-B: 5'TAGCTGAAGAGGCACAGG3' (SEQ ID No: 14) followed by a second amplification step, performed with the pair of primers: NEU-C: 5'GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18) and FIN-D: 5'TCCACCTTCTTCTTCGATT3' (SEQ ID No: 16), to obtain a DNA segment with

2088 base pairs ranging from the residues 6197 to 8284 inclusive and in that the vector in step (c) is a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 6480 to 8263 inclusive, and comprises a single MulI restriction site.

16. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: NEU-A: 5'TAGAAAGAGCAGAAGACAGTGGCAATG3' (SEQ ID No: 17) and FuB: 5' GGTGGTAGCTGAAGAGGCACAGG3' (SEQ ID No: 24), followed by a second amplification step, performed with the pair of primers: NEU-C: 5'GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18) and a mixture of the following primers FuD1: 5'TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No: 26) and FuD2: 5'TCTGTCTTGCTCTCCACCTTCTTCTT3' (SEQ ID No: 27), said mixture being preferently carried out in a ratio comprised between (10%:90%) and (90%-10%) more preferently between (60%:40%) and (40%:60%), to obtain a DNA segment with 2118 base pairs ranging from the residues 6322 to 8440 inclusive and the vector in step c is a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 6480 to 8263 inclusive, and comprises a single MulI restriction site.

17. Analytical method according to claims 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: E00: 5'TAGAAAGAGCAGAAGACAGTGGCAATGA3' (SEQ ID No: 19) and ES8B: 5'CACTTCTCCAATTGTCCCTCA3' (SEQ ID No: 22), followed by a second amplification step, performed with the pair of primers: E20: 5'GGGCCACACATGCCTGTGTACCCACAG3' (SEQ ID No: 21) and E115: 5'AGAAAAATTCCCCTCCACAATTAA3' (SEQ ID No: 22), to obtain a DNA segment with 938 base pairs ranging from the residues 6426 to 7364 inclusive and in that the vector in step (c) is a retroviral vector deleted from the region, coding for the domains ranging from the loop V1 to the loop V3 of the **HIV**-1 envelope ranging from 6617 to 7250 inclusive and comprises a single NheI restriction site.

18. Analytical method according to claims 1, 2, 11 to 17 consisting of determining the susceptibility of an **HIV** virus to a fusion inhibiting compound targeting **HIV**-1 gp41 protein, characterised in that said fusion inhibiting compound is added, possibly at different concentrations, during the culture of the cell host obtained in step (e), before step (1) and in that step (g) comprises the comparison of the expression of the marker gene with and without fusion inhibiting compound targeting **HIV**-1 gp41.

19. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to a compound inhibiting the entry of said **HIV** virus into a target cell, characterised in that said entry inhibiting compound is added, possibly at different concentrations, to the cell host obtained in step (e) before the infection in step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without entry inhibiting compound.

20. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to the inhibitory action of **antibodies**, characterised in that said method is carried out, firstly without **antibodies** and, secondly, with the **antibody**, possibly at different concentrations, said **antibody** being present in step (e), and in that step (g) comprises the comparison of the expression of the marker gene with and without **antibodies**.

21. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the tropism of an **HIV** virus for a cell receptor, characterised in that the infection in step (f) with the viral particles

obtained in step (c) is performed on two separate cell hosts and step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

22. Analytical method according to claim 21 characterised in that one of two cell hosts infected in step (g) expresses the CCR5 receptor and the other expresses the CXCR4 receptor.

23. Analytical method according to claims 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to an inhibiting compound targeting **HIV**-1 co-receptors, characterised in that said inhibiting compound targeting **HIV**-1 co-receptors is added or not, possibly at different concentrations, during the culture step (e), in that the infection in step (f) is performed on two separate cell hosts and in that step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

24. Analytical method according to claims 1, 2, 11, 12 or 17 consisting of analysing the tropism of an **HIV** virus for a cell receptor, characterised in that the infeciton in step (f) with the viral particles obtained in step (c) is performed on two separate cell hosts and step (g) comprises a comparison of the expression of the marker gene by each of the two separate cell hosts.

25. Analytical method according to claims 1, 2, 11, 12 or 17 consisting of analysing the susceptibility of an **HIV** virus to an inhibiting compound targeting **HIV**-1 co-receptors, characterised in that said inhibiting compound targeting **HIV**-1 co-receptors is added, possibly at different concentrations, during the culture in step (d), in that the infection in step (f) with the viral particles in step (e) is performed on two separate cell hosts and in that step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

26. Analytical method according to any of claims 1 to 17 consisting of determining the infectivity or replicative capacity of an **HIV** virus characterised in that step (g) comprises the comparison of the expression of the marker gene by the second cell host infected with the viral particles obtained by applying steps (a) to (f) to a biological specimen from a patient, and the expression of the marker gene by the same second cell host infected with the reference viral particles obtained by applying steps (a) to (f) to a specimen containing a reference virus.

27. Analytical method according to claim 26 characterised in that the reference viral particles from a reference virus are viral particles obtained by applying steps (a) to (f) to a biological specimen from the same patient at an earlier stage or treatment or before said treatment.

28. Analytical method according to claims 1 to 17 consisting of determining the susceptibility of an **HIV** virus to hydroxyurea, characterised in that hydroxyurea is added or not, possibly at different concentrations, either during the culture step (e), or to the second cell host, before the infection of said host in step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without hydroxyurea.

29. Analytical method according to any of claims 1 to 28 characterised in that the culture step (c) is performed during a period ranging from 12 hours to 72 hours, preferentially from 24 hours to 48 hours.

30. A kit for implementing the method according to any of claims 1 to 29 characterised in that it comprises: i) a pair of primers bordering a nucleic acid sequence of the viral genome liable to comprise at least one mutation, ii) a vector comprising the parts of an **HIV** virus genome required for viral replication except for the segment amplified

with the primers defined in (i) and the gene coding for the envelope protein, iii) a second vector comprising a gene coding for envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

31. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 1 and SEQ ID No: 2 SEQ ID No: 3 and SEQ ID No: 4 ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 protease ranging from the residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a single MluI restriction site, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

32. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 5 and SEQ ID No: 7 SEQ ID No: 6 and SEQ ID No: 8 ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 reverse transcriptase ranging from the residues 2618 to 2872 inclusive, and comprising a single MluI restriction site. iii) a pseudotype virus with a gene coding for an envelope protein, vi) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

33. A kit according to claim 30 characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 9 and SEQ ID No: 10 SEQ ID No: 11 and SEQ ID No: 12 ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 integrase ranging from the residues 4228 to 5093 inclusive and the region coding for the viral envelope between the positions 6343 and 7611 inclusive, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

34. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 13 and SEQ ID No: 14 SEQ ID No: 15 and SEQ ID No: 16 ii) a retroviral vector deleted from the entire region coding for the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 7745 to 8263 inclusive, and comprising a single MluI restriction site, iv) a first cell host liable to be infected by an **HIV** virus. v) a secind cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

35. A kit according to claim 30, characterised in that it comprises: i) the sequence primers: (SEQ ID No: 23) and (SEQ ID No: 24) (SEQ ID No: 25) and the mixtuer of primers (SEQ ID No: 26) and (SEQ ID No: 27), ii) a retroviral vector deleted from the entire region coding for the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 7745 to 8263 inclusive, and comprising a single MluI

restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

36. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 17 and SEQ ID No: 14 SEQ ID No: 18 and SEQ ID No: 16 ii) a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 6480 to 8263 inclusive, and comprising a single MulI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated by viral particles, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

37. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 17 and SEQ ID No: 24 SEQ ID No: 18 and SEQ ID No: 26 and SEQ ID No: 27 ii) a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 6480 to 8263 inclusive, and comprising a single MulI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated by viral particles, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

38. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 19 and SEQ ID No: 20 SEQ ID No: 21 and SEQ ID No: 22 ii) a retroviral vector deleted from the region, coding for the domains ranging from the loop V1 to the loop V3 of the **HIV**-1 envelope, ranging from 6617 to 7250 inclusive, and comprising a single NheI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

L16 ANSWER 9 OF 26 USPATFULL on STN
2003:260677 Methods for stable transduction of cells with **hiv**-derived viral vectors.
Humeau, Laurent, Gaithersburg, MD, United States
Han, Wei, Montgomery Village, MD, United States
Lu, Xiaobin, Gaithersburg, MD, United States
Slepushkin, Vladimir, Damascus, MD, United States
Lesher, Mechelle, Frederick, MD, United States
Davis, Brian, Gaithersburg, MD, United States
Chang, Yung-Nien, Cockeysville, MD, United States
Dropulic, Boro, Ellicott City, MD, United States
VIRxSYS Corporation, Gaithersburg, MD, United States (U.S. corporation)
US 6627442 B1 20030930
APPLICATION: US 2000-653088 20000831 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods, as well as compositions related thereto, for the efficient transduction of cells using viral vectors. The efficiency of transduction is increased by contacting the cell to be transduced with one or more molecules that bind the cell surface.

contact with a cell surface binding molecule may occur before, after, or simultaneously with contact between the viral vector and the cell. The transduced vectors may be constructed to express a gene of interest, permitting the transduced cells to be used as therapeutic and prophylactic agents.

CLM What is claimed is:

1. A method for stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells comprising contacting the surface of said cells with both an **HIV** derived lentiviral vector and at least one molecule which binds said cell surface wherein said contacting occurs in vitro or ex vivo and wherein greater than about 90% of the cells are stably transduced after about 14 days.

2. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs before contacting the cells with at least one cell surface binding molecule.

3. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs simultaneously with contacting the cells with at least one cell surface binding molecule.

4. The method of claim 1 wherein said contacting the cells with a lentiviral vector occurs after contacting the cells with at least one cell surface binding molecule.

5. The method of claim 1 where said contacting with a lentiviral vector occurs more than once.

6. The method of claim 1 wherein said cell surface binding molecule is an **antibody**, a ligand or a cell surface molecule.

7. The method of claim 1 wherein said lentiviral vector comprises at least one cis-acting nucleotide sequence derived from the gag, pol, env, vif, **vpr**, vpu, tat or rev genes.

8. The method of claim 7 wherein said sequence is not expressed or is a fragment or a mutant of the gag, pol, env, vif, **vpr**, vpu, tat or rev genes.

9. The method of claim 1 wherein said lentiviral vector is a pseudotyped vector.

10. The method of claim 9 wherein said pseudotyped vector contains the vesicular stomatitis virus G envelope protein.

11. The method of claim 1 wherein said hematopoietic cell is a CD4 positive cell.

12. The method of claim 1 wherein said hematopoietic cell is a lymphocyte.

13. The method of claim 12 wherein said lymphocyte is a CD4 or CD8 positive cell.

14. The method of claim 1 wherein said hematopoietic cell is a CD34 positive cell.

15. The method of claim 14 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or **antibodies** that are cell surface binding analogs of FLT-3 ligand, TPO ligand, or Kit ligand.

16. The method of claim 1 wherein said at least one cell surface binding molecule comprises a molecule selected from FLT-3 ligand, TPO ligand, Kit ligand, or **antibodies** that are cell surface binding analogs of

17. The method of claim 1 wherein the said cell is a dendritic cell or a cell capable of differentiating into a dendritic cell.

18. The method of claim 17 wherein said at least one cell surface binding molecule is selected from compositions comprising GM-CSF, IL-4, and TNF-alpha; GM-CSF and interferon-alpha; or **antibodies** that are cell surface binding analogs of GM-CSF, IL-4, and TNF-alpha; GM-CSF or interferon-alpha.

19. The method of claim 11 wherein said at least one cell surface binding molecule is selected from the group consisting of CD3 **antibodies** and fragments thereof, CD28 **antibodies** and fragments thereof, and combinations of said **antibodies** and fragments thereof.

20. The method of claim 19 wherein said at least one cell surface binding molecule comprises a combination of CD3 and CD28 **antibodies** immobilized on coated beads.

21. The method of claim 3 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

22. The method of claim 21 wherein said conditions comprise further incubation with a cell surface binding molecule or a cytokine.

23. The method of claim 22 wherein said cytokine is interleukin-2.

24. The method of claim 21 wherein said culturing is for about seven days.

25. The method of claim 21 wherein said culturing is for about 14 days.

26. The method of claim 3 wherein said contacting the cells with a lentiviral vector is for about 24 hours and is optionally repeated at least once.

27. The method of claim 1 wherein the lentiviral vector is present at an MOI of less than 500.

28. A method to introduce genetic material into a living subject comprising introduction of a cell transduced by the method of claim 1.

29. The method of claim 4 further comprising culturing the cells under conditions conducive to growth and/or proliferation.

30. The method of claim 1 wherein said contacting occurs ex vivo.

L16 ANSWER 10 OF 26 USPATFULL on STN
2003:231970 Complete genome sequence of a simian immunodeficiency virus from a red-capped mangabey.
Hahn, Beatrice H., Birmingham, AL, UNITED STATES
Gao, Feng, Hoover, AL, UNITED STATES
Shaw, George M., Birmingham, AL, UNITED STATES
Marx, Preston A., Covington, LA, UNITED STATES
Smith, Stephen M., Essex Fells, NJ, UNITED STATES
Georges-Courbot, Marie Claude, Paris XV, FRANCE
Lu, Chang Yong, Forest Hills, NY, UNITED STATES
UAB Research Foundation (U.S. corporation)
US 2003162170 A1 20030828
APPLICATION: US 2003-369294 A1 20030218 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The nucleotide sequence and deduced amino acid sequences of the complete genome of a simian immunodeficiency virus isolate from a red-capped

[illegible] peptides encoded by and/or derived from these sequences and their use in diagnostic methods and as immunogens.

CLM What is claimed is:

1. A nucleic acid comprising the nucleotide sequence of the genome of the simian immunodeficiency virus isolate SIVrcm shown in SEQ ID NO:1 or a complementary sequence thereof.

2. The nucleic acid of claim 1, wherein said nucleic acid comprises a nucleotide sequence of at least 12 contiguous bases of said nucleic acid or a complementary sequence thereof.

3. The nucleic acid of claim 1, wherein said nucleic acid comprises a nucleotide sequence of a LTR of said nucleic acid or a complementary sequence thereof.

4. The nucleic acid of claim 1, wherein said nucleic acid encodes a polypeptide selected from the group consisting of Gag, Pol, Vif, **Vpr**, Env, Tat, Rev, Nef and Vpx of SIVrcm.

5. The nucleic acid of claim 1, wherein said nucleic acid has a nucleotide sequence which is derived from SEQ ID NO:1.

6. A vector comprising the nucleic acid of claim 1.

7. A cell comprising the nucleic acid of claim 1.

8. A cell comprising the vector of claim 6.

9. A composition comprising the nucleic acid of claim 1 and a physiologically acceptable carrier.

10. A method for producing a polypeptide encoded by the nucleic acid of claim 1, comprising the step of growing a cell comprising the nucleic acid of claim 1 under conditions such that the encoded polypeptide is produced.

11. The method of claim 10, wherein said polypeptide comprises a contiguous sequence of at least 13 amino acids.

12. A composition comprising the polypeptide produced by the method of claim 10 and a physiologically acceptable carrier.

13. A method for producing a polypeptide encoded by the nucleic acid of claim 1, comprising the step of growing a cell comprising a vector, said vector comprising the nucleic acid of claim 1, under conditions such that the encoded polypeptide is produced.

14. The method of claim 13, wherein said polypeptide comprises a contiguous sequence of at least 13 amino acids.

15. A composition comprising the polypeptide produced by the method of claim 13 and a physiologically acceptable carrier.

16. A method of inducing serum **antibodies** that bind at least one polypeptide encoded by the nucleic acid of claim 1, said method comprising: administering to a mammal, in a physiologically acceptable carrier, an amount of said encoded polypeptide sufficient to elicit production of said **antibodies**.

17. An anti-SIVrcm **antibody** made by the method of claim 16.

18. A composition comprising an **antibody** according to claim 17 and a physiologically acceptable carrier.

19. A method for detecting the presence of SIVrcm in a sample comprising

contacting said sample with the **antibody** of claim 17 under conditions that allow the formation of an **antibody**-antigen complex and detecting said complex.

20. A kit for detecting the presence of SIVrcm in a sample comprising an **antibody** of claim 17.

21. A method of inducing serum **antibodies** that bind at least one polypeptide encoded by the nucleic acid of claim 1, said method comprising: administering to a mammal, in a physiologically acceptable carrier, the nucleic acid of claim 1 encoding said polypeptide and which produces an immunologically sufficient amount of the encoded polypeptide to elicit said **antibodies**.

22. An **antibody** to SIVrcm made by the method of claim 21.

23. A composition comprising an **antibody** according to claim 22, and a physiologically acceptable carrier.

24. A method for detecting the presence of SIVrcm in a sample comprising contacting said sample with the **antibody** of claim 22 under conditions that allow the formation of an **antibody**-antigen complex and detecting said complex.

25. A kit for detecting the presence of SIVrcm in a sample comprising an **antibody** of claim 22.

26. A method for detecting the presence of **antibodies** to SIVrcm in a sample comprising contacting said sample with a polypeptide encoded by the nucleic acid of claim 1 under conditions that allow the formation of an **antibody**-antigen complex and detecting the complex.

27. A method for detecting the presence of SIVrcm in a sample comprising contacting said sample with the nucleic acid of claim 1 and detecting said nucleic acid bound to the genomic DNA, mRNA or cDNA of the SIVrcm virus.

28. A kit for detecting the presence of SIVrcm in a sample comprising the nucleic acid of claim 1.

29. A nucleic acid probe comprising a sequence of at least 19 contiguous nucleotides derived from the nucleic acid of claim 1, or the complementary sequence thereof.

30. A composition comprising a nucleic acid according to claim 29.

31. A method of detecting the presence of SIVrcm in a biological sample comprising: a.) contacting the nucleic acid of the biological sample with the nucleic acid probe of claim 29; and b.) detecting the presence or absence of complexes formed between said nucleic acid of the biological sample and said nucleic acid probe.

32. A method of detecting the presence of SIVrcm in a biological sample comprising: a). contacting said biological sample with at least two nucleic acid probes of claim 29; b). amplifying the RNA of the biological sample via reverse transcription-polymerase chain reaction to produce amplification products; and c.) detecting the presence or absence of amplification products.

33. A method for analyzing a first nucleotide sequence comprising comparing the nucleotide sequence of claim 1 with said first sequence.

L16 ANSWER 11 OF 26 USPATFULL on STN
2003:225881 Cell lines with latent immunodeficiency virus and methods of use thereof.

Jordan, Albert, San Francisco, CA, UNITED STATES
US 2003157693 A1 20030821
APPLICATION: US 2002-323463 A1 20021218 (10)
PRIORITY: US 2001-341727P 20011219 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides isolated cells that comprise, integrated into the genome of the cell, a transcription-competent immunodeficiency virus or a transcription-competent immunodeficiency virus-based retroviral vector. Under basal in vitro culture conditions, the immunodeficiency virus is latent, and the expression of the latent immunodeficiency virus can be reactivated. The invention further provides methods of making a subject cell. The invention further provides screening methods for identifying agents that activate a latent immunodeficiency virus; and screening method for identifying agents that block reactivation of latent immunodeficiency virus expression in response to T cell activation signals. The invention further provides agents identified in the subject screening assays. The invention further provides methods of treating an immunodeficiency virus infection.

CLM What is claimed is:

1. An isolated cell that comprises, integrated into the genome of the cell, a recombinant transcription-competent immunodeficiency virus-based vector, wherein, under basal in vitro culture conditions, the immunodeficiency virus is latent, and wherein expression of the latent immunodeficiency virus can be reactivated.

2. The cell of claim 1, wherein said cell is an immortalized cell line.

3. The cell of claim 1, wherein said cell is a T lymphoid cell.

4. The cell of claim 1, wherein said immunodeficiency virus is **human immunodeficiency virus** (**HIV**).

5. The cell of claim 4, wherein said immunodeficiency virus is **HIV**-1.

6. A method of making an immortalized cell that comprises, integrated into the genome of the cell, a recombinant, transcription-competent **human immunodeficiency virus** (**HIV**) vector, wherein, under basal in vitro culture conditions, the **HIV** is latent, and wherein expression of the latent **HIV** can be reactivated, the method comprising: a) introducing into population of immortalized cells in vitro a recombinant, transcription-competent **HIV** that comprises a nucleotide sequence encoding a selectable marker operably linked to a promoter; and b) selecting a cell population that comprises the recombinant **HIV** integrated into the genome of the cell, and that does not produce the detectable marker.

7. The method of claim 6, further comprising cloning a cell from the selected cell population.

8. The method of claim 6, wherein step (b) results in a first selected cell population, and the method further comprises the steps of: c) contacting said first selected cell population with an agent that activates **HIV** transcription; d) selecting a second population of cells from the first selected population, which second selected population produces the selectable marker.

9. The method of claim 8, wherein said agent is selected from the group consisting of an activator of NF-κB, an agent that cross-links cell-surface T-cell receptor, and an inhibitor of hi stone deacetylase.

10. The method of claim 9, wherein said activating agent is selected from the group consisting of phytohemagglutinin, tetradecanoyl phorbol acetate, TNFα, an anti-CD3 **antibody**, and trichostatin A.

11. An isolated immortalized cell that comprises, integrated into the genome of the cell, a recombinant transcription-competent **human immunodeficiency virus** (**HIV**) vector that comprises a nucleotide sequence encoding a selectable marker operably linked to a promoter, wherein, under basal in vitro culture conditions, the **HIV** is latent, and wherein expression of the latent **HIV** can be reactivated.

12. A method of identifying an agent that activates a latent **human immunodeficiency virus** (**HIV**), the method comprising: a) contacting the cell according to claim 11 with a test agent; and b) determining the effect, if any, of the test agent on production of the detectable marker, wherein production of the detectable marker indicates that the test agent activates a latent **HIV**.

13. The method of claim 12, wherein said detectable marker is a fluorescent protein, and said determining is detection of fluorescence.

14. A composition comprising an agent identified by the method of claim 12; and a pharmaceutically acceptable excipient.

15. A method of reducing the number of cells containing a latent **human immunodeficiency virus** in an individual, the method comprising: administering to the individual an effective amount of the composition of claim 14.

16. A method of treating a **human immunodeficiency virus** infection in an individual, the method comprising: administering to an individual an effective amount of the composition of claim 14; and administering to the individual an effective amount of an agent that inhibits an immunodeficiency virus function selected from the group consisting of viral replication, viral protease activity, viral reverse transcriptase activity, viral entry into a cell, viral integrase activity, viral Rev activity, viral Tat activity, viral Nef activity, viral **Vpr** activity, viral Vpu activity, and viral Vif activity.

L16 ANSWER 12 OF 26 USPATFULL on STN
2003:225848 Non-M, non-O **HIV**-1 strains, fragments and uses.
Mauclere, Phillippe, Bordeaux, FRANCE
Loussert-Ajaka, Ibtissam, Sartrouville, FRANCE
Simon, Francois, Paris, FRANCE
Saragosti, Sentob, Billancourt, FRANCE
Barre-Sinoussi, Francoise, Moulimeaux, FRANCE
Institute National de la Sante et de La Recherche Medicale-Inserm (non-U.S. corporation)
US 2003157660 A1 20030821
APPLICATION: US 2002-301661 A1 20021122 (10)
PRIORITY: FR 1996-15087 19961209
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Retroviral strains of the non-M, non-O **HIV**-1 group, in particular a strain designated YBF30, its fragments and also its uses as a diagnostic reagent and as an immunogenic agent.

The **HIV**-1 viruses which differ both from the M group and the O group exhibit the following characteristics:

little or no serological reactivity with regard to the proteins of the M and O groups and strong serological reactivity with regard to the proteins which are derived from the strain YBF30 according to the invention or the strain CPZGAB SIV;

absence of genomic amplification when using primers from the env and gag regions of the M and O **HIV**-1 groups;

genomic amplification in the presence of primers which are derived from

the YBF30 strain according to the invention, and

homology of the products of the envelope gene which is greater than 70% with regard to the YBF30 strain.

CLM What is claimed is:

1) Non-M, non-O **HIV**-1 strain which exhibits the morphological and immunological characteristics of the retrovirus which was deposited on Jul. 2, 1996 under number I-1753 (designated YBF30) in the Collection Nationale de Cultures de Microorganismes (National Collection of Microorganism Cultures) kept by the Pasteur Institute.

2) Nucleic acid sequences, characterized in that they are derived from the strain according to claim 1.

3) Nucleic acid sequence according to claim 2, characterized in that it is selected from the group consisting of the following sequences: the complete nucleotide sequence of the strain according to claim 1 (SEQ ID No.1) as well as nucleic acid fragments which are derived from the said strain: (SEQ ID No.2), (SEQ ID No.3), (SEQ ID No.5), (SEQ ID No.7), (SEQ ID No.9), (SEQ ID No.11), (SEQ ID No.13), (SEQ ID No.15), (SEQ ID No.17), (SEQ ID No.19) and the sequences SEQ ID No. 21-57, and also any sequence which is not identical to one of the above nucleotide sequences, or is not complementary to one of these sequences, but is nevertheless capable of hybridizing with a nucleic acid sequence which is derived from a non-M, non-O **HIV**-1 virus.

4) Oligonucleotide, characterized in that it is selected from the sequences SEQ ID No. 21 to 57, and in that it is capable of being used as a primer or as a probe for detecting an **HIV**-1 according to claim 1 or claim 5.

5) **HIV**-1 viruses, characterized in that they differ both from the M group and from the O group and exhibit the following characteristics: little or no serological reactivity with regard to proteins of the M and O groups and strong serological reactivity with regard to proteins which are derived from the YBF30 strain according to claim 1 or the CPZGAB SIV strain; absence of genomic amplification when using primers from the env and gag regions of the **HIV**-1 viruses of the M and O groups; genomic amplification in the presence of the primers which are derived from the YBF30 strain according to claim 4; and homology of the products of the envelope gene which is greater than 70% with regard to the YBF30 strain.

6) Method for diagnosing in vitro an **HIV**-1 virus of the non-M, non-O group by means of hybridization and/or gene amplification, which method is carried out using a biological sample (serum or circulating lymphocyte) and is characterized in that it comprises: a step of extracting the nucleic acid which is to be detected and which belongs to the genome of the virus, which virus may possibly be present in the biological sample, and, where appropriate, a step of treating the nucleic acid using a reverse transcriptase, if this nucleic acid is in RNA form, at least one cycle comprising the steps of denaturing the nucleic acid, of hybridizing with at least one sequence according to claim 3 or claim 4 and, where appropriate, extending the hybrid, which has been formed, in the presence of suitable reagents (polymerizing agent, such as DNA polymerase and dNTP), and a step of detecting the possible presence of the nucleic acid belonging to the genome of a virus of the non-M, non-O **HIV**-1 group type.

7) Peptide, characterized in that it can be expressed by a non-M, non-O **HIV**-1 strain according to claim 1 or claim 5 or using a nucleotide sequence according to claim 3, and in that it is capable (1) of being recognized by **antibodies** which are induced by a non-M, non-O **HIV**-1 virus according to claim 1 or claim 5, or a variant of this virus, and which are present in a biological sample which is obtained following an infection with a non-M, non-O **HIV**-1 strain, and/or (2) of inducing the

production of anti-non-M, non-O **HIV**-1 **antibodies**.

8) Peptide according to claim 7, characterized in that it is selected from that which is expressed by the gag gene (SEQ ID No. 4), that which is expressed by the pol gene (SEQ ID No. 6), that which is expressed by the vif gene (SEQ ID No. 8), that which is expressed by the **vpr** gene (SEQ ID No. 10), that which is expressed by the vpu gene (SEQ ID No. 12), that which is expressed by the tat gene (SEQ ID No. 14), that which is expressed by the rev gene (SEQ ID No. 16), that which is expressed by the env gene (SEQ ID No. 18) or one of its fragments such as a fragment of the V3 loop region (SEQ ID No. 58), and that which is expressed by the nef gene (SEQ ID No. 20), or a fragment of these peptides which are capable of recognizing the **antibodies** which are produced during an infection with an **HIV**-1 virus according to claim 1 or claim 5.

9) Immunogenic compositions which comprise one or more translation products of the nucleotide sequences according to claim 3 and/or one of the peptides according to claim 7 or claim 8.

10) **Antibodies** which are directed against one or more of the peptides according to claim 7 or claim 8.

11) Method for the in-vitro diagnosis of a non-M, non-O **HIV**-1 virus, characterized in that it comprises bringing into contact a biological sample, which has been withdrawn from a patient, with **antibodies** according to claim 10, which may possibly be combined with anti-CPZGAB SIV **antibodies**, and detecting the immunological complexes which are formed between the **HIV**-1 antigens, which may possibly be present in the biological sample, and the said **antibodies**.

L16 ANSWER 13 OF 26 USPATFULL on STN
2003:213629 Reference clones and sequences for non-subtype B isolates of **human immunodeficiency virus** type 1.
Hahn, Beatrice H., Birmingham, AL, UNITED STATES
Shaw, George M., Birmingham, AL, UNITED STATES
Gao, Feng, Hoover, AL, UNITED STATES
UAB Research Foundation (U.S. corporation)
US 2003148266 A1 20030807
APPLICATION: US 2002-290579 A1 20021108 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The nucleotide sequences of the genomes of eleven molecular clones for non-subtype B isolates of **human immunodeficiency virus** type 1 are disclosed. The invention relates to the nucleic acids and peptides encoded by and/or derived from these sequences and their use in diagnostic methods and as immunogens.

CLM What is claimed is:
1. A nucleic acid comprising the nucleotide sequence of the genome of a non-subtype B **HIV**-1 virus, wherein said nucleotide sequence is selected from sequences having SEQ ID NOS: 1 to 11 or a complementary sequence thereof.

2. The nucleic acid of claim 1, wherein said nucleic acid comprises a nucleotide sequence of at least 12 contiguous bases of said nucleic acid or a complementary sequence thereof.

3. The nucleic acid of claim 1, wherein said nucleic acid comprises a nucleotide sequence of a LTR of said nucleic acid or a complementary sequence thereof.

4. The nucleic acid of claim 1, wherein said nucleic acid encodes a polypeptide selected from the group consisting of Gag, Pol, Vif, **Vpr**, Env, Tat, Rev, Nef and Vpu, wherein the nucleic acid is from the genome of a non-subtype B **HIV**-1 virus selected from the group consisting of 92RW009.6, 92NG003.1, 92NG083.2, 93BR020.1, 93BR029.4, 90CF056.1,

complementary sequence thereof.

5. A vector comprising a nucleic acid of claim 1.

6. A cell comprising a nucleic acid of claim 1.

7. A cell comprising the vector of claim 5.

8. A polypeptide encoded by a nucleic acid of claim 1.

9. The polypeptide of claim 8 comprising a contiguous sequence of at least 13 amino acids.

10. A method for producing a polypeptide of claim 8, comprising the step of growing a cell under conditions such that the encoded polypeptide is produced, said cell comprising the nucleic acid of the polypeptide of claim 8.

11. A method for producing a polypeptide of claim 8, comprising the step of growing a cell under conditions such that the encoded polypeptide is produced, said cell comprising a vector having the nucleic acid of the polypeptide of claim 8.

12. A method of inducing serum **antibodies** that bind at least one polypeptide of claim 8, comprising the step of administering to a mammal, in a physiologically acceptable carrier, an amount of polypeptide of claim 8 sufficient to elicit production of said **antibodies**.

13. A method for detecting the presence of **antibodies** to a non-subtype B **HIV**-1 virus in a sample comprising the step of contacting said sample with said polypeptide of claim 8 under conditions that allow the formation of an **antibody**-antigen complex and detecting the complex.

14. An **antibody** to a non-subtype B **HIV**-1 virus, said **antibody** binding at least one polypeptide of claim 8, wherein said **antibody** is made by administering to a mammal an amount of said polypeptide in a physiological carrier sufficient to elicit production of said **antibody**.

15. A composition comprising the **antibody** of claim 14, and a physiologically acceptable carrier.

16. A method for detecting the presence of a non-subtype B **HIV**-1 virus in a sample comprising the step of contacting said sample with the **antibody** of claim 14 under conditions that allow the formation of an **antibody**-antigen complex and detecting said complex.

17. A kit for detecting the presence of a non-subtype B **HIV**-1 virus in a sample comprising the **antibody** of claim 14.

18. A method of inducing serum **antibodies** that bind at least one polypeptide of claim 8, comprising the step of administering to a mammal, in a physiologically acceptable carrier, the nucleic acid which encodes said polypeptide(s) and which produces an immunologically sufficient amount of the encoded polypeptide to elicit said **antibodies**.

19. An **antibody** to a non-subtype B **HIV**-1 virus, said **antibody** binding at least one polypeptide of claim 8, wherein said **antibody** is made by administering to a mammal the nucleic acid encoding said polypeptide(s) in a physiologically acceptable carrier, wherein said nucleic acid produces an immunologically sufficient amount of the encoded polypeptide(s) to elicit said **antibody**.

20. A composition comprising the **antibody** of claim 19, and a physiologically acceptable carrier.

21. A method for detecting the presence of a non-subtype B **HIV**-1 virus in a sample comprising the step of contacting said sample with an **antibody** of claim 19 under conditions that allow the formation of an **antibody**-antigen complex and detecting said complex.

22. A kit for detecting the presence of a non-subtype B **HIV**-1 virus in a sample comprising the **antibody** of claim 19.

23. A method for detecting the presence of a non-subtype B **HIV**-1 virus in a sample comprising the steps of: contacting said sample with a nucleic of claim 1; and detecting said nucleic acid bound to the genomic DNA, mRNA or cDNA of the non-subtype B **HIV**-1 virus.

24. A nucleic acid probe comprising a sequence of at least 19 contiguous nucleotides of a nucleic acid of claim 1.

25. A method of detecting the presence of a non-subtype B **HIV**-1 virus in a biological sample comprising the steps of: contacting the nucleic acid of the biological sample with the nucleic acid probe of claim 24; and detecting the presence or absence of complexes formed between said nucleic acid of the biological sample and said nucleic acid probe.

26. A method of detecting the presence of a non-subtype B **HIV**-1 virus in a biological sample comprising the steps of: contacting said biological sample with at least two nucleic acid probes of claim 26; amplifying the RNA of the biological sample via reverse transcription-polymerase chain reaction to produce amplification products; and detecting the presence or absence of amplification products.

27. A composition comprising the nucleic acid probe of claim 24.

28. A method for analyzing a first nucleotide sequence comprising comparing the nucleotide sequence of any one of the nucleotide sequences having SEQ ID NOS: 1 to 11 with said first sequence.

29. A method for analyzing a first amino acid sequence comprising comparing the amino acid sequence of any one of the amino acid sequences set forth in FIGS. 14-22 with said first sequence.

L16 ANSWER 14 OF 26 USPATFULL on STN
2003:200475 Method of vaccination through serotype rotation.
Wang, Danher, Mt. Pleasant, SC, UNITED STATES
US 2003138459 A1 20030724
APPLICATION: US 2003-286332 A1 20030317 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Recombinant adenovirus and methods of administration to a host are provided for eliciting immune response of the host to various pathogens. In one aspect of the invention, a vaccination method is provided for enhancing immunity of the host to the pathogen through rotation of the serotypes of the recombinant adenoviruses administered to the host. The method comprises administering to the host a first recombinant adenovirus comprising a first antigen sequence heterologous to native adenovirus and encoding a first viral antigen from the first pathogenic virus, expression of the first viral antigen by the first recombinant adenovirus eliciting an immune response directed against the first viral antigen in a host upon infection of the host by the first recombinant adenovirus; and administering to the host a second recombinant adenovirus comprising a second antigen sequence heterologous to native adenovirus and encoding a second viral antigen from the second pathogenic virus, expression of the second viral antigen by the second recombinant adenovirus eliciting an immune response directed against the second viral antigen in a host upon infection of the host by the first

recombinant adenovirus. The serotype of the second recombinant adenovirus is different from that of the first recombinant adenovirus, or certain region(s) in the backbone of the second recombinant adenovirus (e.g., the fiber region) is of different serotype from the corresponding region(s) in the backbone of the first recombinant adenovirus.

CLM What is claimed is:

1. A method for enhancing the immunity of a host to infection of a first and second pathogenic virus, comprising: administering to the host a first recombinant adenovirus comprising a first antigen sequence heterologous to native adenovirus and encoding a first viral antigen from the first pathogenic virus, expression of the first viral antigen by the first recombinant adenovirus eliciting an immune response directed against the first viral antigen in a host upon infection of the host by the first recombinant adenovirus; and administering to the host a second recombinant adenovirus comprising a second antigen sequence heterologous to native adenovirus and encoding a second viral antigen from the second pathogenic virus, expression of the second viral antigen by the second recombinant adenovirus eliciting an immune response directed against the second viral antigen in a host upon infection of the host by the first recombinant adenovirus.

2. The method of claim 1, wherein administering to the host the first or second recombinant adenovirus is performed intramuscularly, intratracheally, subcutaneously, intranasally, intradermally, rectally, orally or parentally.

3. The method of claim 1, wherein the serotype of the first recombinant adenovirus is adenovirus serotype 5, and the serotype of the second recombinant adenovirus is selected from the group consisting of adenovirus serotype 1-4 and 6-51.

4. The method of claim 1, wherein the first viral antigen encoded by the first recombinant adenovirus is the same as the second viral antigen encoded by the second recombinant adenovirus.

5. The method of claim 1, wherein the first viral antigen encoded by the first recombinant adenovirus is different from the second viral antigen encoded by the second recombinant adenovirus.

6. The method of claim 1, wherein the second recombinant adenovirus has the same adenoviral backbone as the first one except that the fiber region of the second recombinant adenovirus is of different serotype than that in the first recombinant adenovirus.

7. The method of claim 1, wherein the second recombinant adenovirus has the same adenoviral backbone as the first one except that the knob, shaft, or penton base domain of the fiber region of the second recombinant adenovirus is of different serotype than the corresponding one in the first recombinant adenovirus.

8. The method of claim 1, wherein the second recombinant adenovirus has the same adenoviral backbone as the first one except that the knob domain of the fiber region of the second recombinant adenovirus is of different serotype than the corresponding one in the first recombinant adenovirus.

9. The method of claim 1, wherein the second recombinant adenovirus is administered to the host at least one week post the administration of the first recombinant adenovirus.

10. The method of claim 1, wherein the first or second recombinant adenovirus is replication-incompetent.

11. The method of claim 1, wherein the first recombinant virus further comprises a third antigen sequence heterologous to native adenovirus and

encoding a third viral antigen from the first or second pathogenic virus.

12. The method of claim 11, wherein the first and third antigen sequences are positioned in the E1 and E3 or E4 region of the native progenitor of the first recombinant adenovirus, respectively.

13. The method of claim 11, wherein the first and third antigen sequences are expressed bicistronically by the same promoter.

14. The method of claim 13, wherein the first and second antigen sequences are expressed bicistronically via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.

15. The method of claim 1, wherein the first recombinant virus further comprises a third antigen sequence heterologous to native adenovirus and encoding a third viral antigen from the first or second pathogenic virus, and the second recombinant virus further comprises a fourth antigen sequence heterologous to native adenovirus and encoding a fourth viral antigen from the first or second pathogenic virus.

16. The method of claim 15, wherein the first and third antigen sequences are positioned in the E1 and E3 or E4 region of the native progenitor of the first recombinant adenovirus, respectively; and the second and fourth antigen sequence are positioned in the E1 and E3 or E4 region of the native progenitor of the second recombinant adenovirus, respectively.

17. The method of claim 16, wherein the first and third antigen sequences are expressed bicistronically by the same promoter, or the second and fourth antigen sequences are expressed bicistronically by the same promoter,

18. The method of claim 17, wherein the bicistronic expression is via an internal ribosomal entry site or via a splicing donor-acceptor mechanism.

19. The method of claim 1, wherein the first and the second pathogenic viruses are the same.

20. The method of claim 1, wherein the first and the second pathogenic viruses are of the same type but of different subtype or clade.

21. The method of claim 1, wherein the first and the second pathogenic viruses are different types of the same virus.

22. The method of claim 1, wherein the first and the second pathogenic viruses are different viruses.

23. The method of claim 1, wherein the first or second pathogenic virus is a **human immunodeficiency virus**.

24. The method of claim 23, wherein the first or second viral antigen is an **HIV** surface, core/capsid, regulatory, enzyme or accessory protein.

25. The method of claim 23, wherein the first or second viral antigen is selected from the group consisting of **HIV** gp120, gp41, Gag, p17, p24, p2, p7, p1, p6, Tat, Rev, PR, RT, IN, Vif, **Vpr**, Vpx, Vpu and Nef.

26. The method of claim 1, wherein the first or second pathogenic virus is influenza virus.

27. The method of claim 26, wherein the first or second viral antigen is a glycoprotein of the influenza virus.

28. The method of claim 27, wherein the first or second viral antigen is

29. The method of claim 1, wherein the first or second pathogenic virus is Ebola virus.

30. The method of claim 29, wherein the first or second viral antigen is an Ebola glycoprotein.

31. The method of claim 30, wherein the first or second viral antigen is Ebola GP1 or GP2 protein.

32. The method of claim 31, wherein the first or second viral antigen is an Ebola nucleocapsid protein.

33. The method of claim 1, wherein the first or second pathogenic virus is Marburg virus.

34. The method of claim 33, wherein the first or second viral antigen is a Marburg glycoprotein.

35. The method of claim 26, wherein the first or second viral antigen is a Marburg nucleocapsid protein.

36. The method of claim 1, wherein the first or second pathogenic virus is Arbovirus.

37. The method of claim 36, wherein the first or second viral antigen is Arbovirus glycoprotein.

38. The method of claim 1, wherein the first or second pathogenic virus is hepatitis virus.

39. The method of claim 38, wherein the hepatitis virus is hepatitis A, B, C, D or E virus.

40. The method of claim 38, wherein the first or second viral antigen is surface antigen or core protein of hepatitis B virus.

41. The method of claim 40, wherein the first or second viral antigen is SHBsAg, MHBsAg, or LHBsAg of hepatitis B virus.

42. The method of claim 38, wherein the first or second viral antigen is a surface antigen or core protein of hepatitis C virus.

43. The method of claim 42, wherein the first or second viral antigen is NS3, NS4 or NS5 antigen of hepatitis C virus.

44. The method of claim 1, wherein the first or second pathogenic virus is respiratory syncytial virus.

45. The method of claim 44, wherein the first or second viral antigen is a glycoprotein or a fusion protein of respiratory syncytial virus

46. The method of claim 1, wherein the first or second pathogenic virus is herpes simplex virus.

47. The method of claim 46, wherein the first or second pathogenic virus is herpes simplex virus type-1 or type-2.

48. The method of claim 46, wherein the first or second viral antigen is glycoprotein D from herpes simplex virus type-2.

49. The method of claim 1, wherein the first or second pathogenic virus is human papilloma virus.

50. The method of claim 49, wherein the first or second viral antigen is

26 or 27 of human papilloma virus.

51. The method of claim 1, wherein the first or second viral antigen is a full-length antigenic **viral protein** or a portion of the antigenic **viral protein** that contains the predominant antigen, neutralizing antigen, or epitope of the first or second pathogenic virus.

52. The method of claim 1, wherein the first or second viral antigen is a modified antigen that is mutated from a glycoprotein of the first or second pathogenic virus such that the first or second viral antigen is rendered non-functional as a viral component but retains its antigenicity.

53. The method of claim 52, wherein the modification of first or second viral antigen includes deletions in the proteolytic cleavage site of the glycoprotein, and duplications and rearrangement of immunosuppressive peptide regions of the glycoprotein.

54. The method of claim 1, wherein the first or second recombinant adenovirus further comprises: an immuno-stimulator sequence that is heterologous to native adenovirus and encodes an immuno-stimulator.

55. The method of claim 54, wherein the immuno-stimulator is a cytokine.

56. The method of claim 55, wherein the cytokine is selected from the group consisting of interleukin-2, interleukin-4, interleukin-12, β-interferon, λ-interferon, γ-interferon, granulocyte colony stimulating factor, and granulocyte-macrophage colony stimulating factor.

57. The method of claim 1, further comprising: harvesting serum from the host after the administration of the first and second recombinant adenovirus.

58. The method of claim 57, wherein the host is a human or a non-human primate.

59. The method of claim 57, further comprising: storing the serum for at least 12 hour; and then administering the serum to the host or another host.

60. The method of claim 59, wherein the other host is a human or a non-human primate.

61. The method of claim 1, further comprising: isolating **antibody** against the first or second viral antigen from the host after the administration of the first and second recombinant adenovirus; and then administering the **antibody** to host or another host.

62. The method of claim 61, wherein the host or the other host is a human or a non-human primate.

L16 ANSWER 15 OF 26 USPATFULL on STN
2003:195006 SIV derived lentiviral vector systems.
Planelles, Vicente, Salt Lake City, UT, UNITED STATES
University of Rochester (U.S. corporation)
US 2003134817 A1 20030717
APPLICATION: US 2002-304988 A1 20021126 (10)
PRIORITY: US 2000-207916P 20000530 (60)
US 2001-287433P 20010430 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An SIV-derived vector system for transferring a nucleic acid sequence encoding a target molecule to a host cell is described. The vector system comprises a transfer vector containing the nucleic acid segment

of interest, a packaging vessel which is deleted for at least one of the accessory proteins (vif, **vpr**, vpx, and/or nef), and an env vector containing an envelope protein which is not an SIV envelope protein. In one embodiment the vector system is Rev-independent.

CLM What is claimed is:

1. A Simian Immunodeficiency Virus (SIV)-derived vector system for transferring a nucleic acid encoding a target molecule to a host cell, comprising: (a) a transfer vector containing a nucleic acid sequence encoding a target molecule wherein the nucleic acid sequence is operably linked to a promoter and a SIV packaging sequence including the portion of the SIV long terminal repeat (LTR) sequences necessary to package the SIV RNA into the SIV virion; (b) a packaging vector derived from an SIV strain and which has at least one accessory gene deleted, which further contains a SIV gag gene encoding a gag protein, wherein the gag gene is operably linked to a promoter and a polyadenylation sequence; (c) an env vector containing an env gene encoding a functional envelope protein from a virus other than a lentivirus, wherein the env gene is operably linked to a promoter and a polyadenylation sequence; and (d) a SIV pol gene encoding a pol protein on one of the first two vectors or on at least a third vector, wherein said lentiviral pol gene is operably linked to a promoter and a polyadenylation sequence; wherein only said transfer vector contains said SIV packaging segment to effectively package lentiviral RNA; and wherein the SIV proteins and the envelope protein when expressed in combination form a SIV virion containing an envelope protein around a SIV capsid.

2. The vector system of claim 1, wherein the packaging vector has at least one of the SIV accessory genes vif, **vpr**, vpx and nef deleted.

3. The vector system of claims 1 or 2, wherein the SIV RRE has been deleted.

4. The vector system of claim 3, wherein a transporter element is used in place of RRE.

5. The vector system of claim 4, wherein the transporter element is a post-transcriptional control element in spleen necrosis vector LTR present in the packaging virus at the 5' end.

6. The vector system of claim 1, wherein the env gene is vesicular stomatitic virus-G protein.

7. The vector system of claim 1, wherein the target molecule is operably linked to an inducible promoter.

8. The vector system of claim 1, wherein the target molecule is an antisense molecule, a ribozyme, an **antibody**, a receptor, a cytokine, an angiogenesis modulation or a growth hormone.

9. The vector system of claim 8, wherein the target molecule is a ribozyme directed to a **human immunodeficiency virus**.

10. The vector system of claim 8, wherein the ribozyme or antisense molecule is capable of transplicing.

11. The vector system of claim 1, wherein the env gene encodes an envelope protein that targets an endocytic compartment.

12. A host cell transfected by the vector system of claim 1.

L16 ANSWER 16 OF 26 USPATFULL on STN

2003:152956 Identification of peptides that facilitate uptake and cytoplasmic and/or nuclear transport of proteins, DNA and viruses.

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US 2003104622 A1 20030605
APPLICATION: US 2002-75869 A1 20020213 (10)
PRIORITY: US 1999-151980P 19990901 (60)
US 2000-188944P 20000313 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to internalizing peptides which facilitate the uptake and transport of cargo into the cytoplasm and nuclei of cells as well as methods for the identification of such peptides. The internalizing peptides of the present invention are selected for their ability to efficiently internalize cargo into a wide variety of cell types both in vivo and in vitro. The method for identification of the internalizing peptides of the present invention comprises incubating a target cell with a peptide display library, isolating peptides with internalization characteristics and determining the ability of said peptide to internalize cargo into a cell.
CLM What is claimed is:

1. A peptide having an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DP ARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

2. The peptide of claim 1 wherein said peptide facilitates cellular internalization of a cargo linked thereto.

3. The peptide of claim 2 wherein the peptide has the amino acid sequence TLPSPLALLTVH (SEQ ID NO:59).

4. The peptide of claim 2 wherein the peptide has the amino acid sequence SVSVGMKPSPRP (SEQ ID NO:86).

5. The peptide of claim 1 wherein the peptide provides for nuclear translocation in a target cell.

6. A peptide-cargo complex comprising a peptide and a cargo wherein the peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

7. The peptide-cargo complex of claim 6 wherein the cargo is selected from the group consisting of a polynucleotide, a polypeptide, a small molecule, a virus, a modified virus, a viral vector, and a plasmid.

8. The peptide-cargo complex of claim 6 wherein the cargo is a virus selected from the group consisting of adenovirus, adeno-associated virus, herpes simplex virus, and retrovirus.

9. The peptide-cargo complex of claim 6 wherein the cargo is selected from the group consisting of therapeutic proteins, suicide proteins, tumor suppressor proteins, transcription factors, kinase inhibitors, kinases, cell cycle regulatory proteins, apoptotic proteins, anti-apoptotic proteins, viral antigens, cellular antigens, differentiation factors, immortalization factors, toxins, **antibodies** and inhibitors of NF-κB.

10. The peptide-cargo complex of claim 6 wherein the peptide facilitates

cellular internalization of cargo linked thereto.

11. The peptide-cargo complex of claim 6 wherein the peptide provides for nuclear translocation of said peptide-cargo complex in a target cell.

12. The peptide-cargo complex of claim 6 wherein the peptide is biotinylated and the cargo is avidin labeled.

13. The peptide-cargo complex of claim 9, wherein the cargo is an apoptotic protein selected from the group consisting of p53, caspase-3, HSV thymidine kinase and an antimicrobial peptide.

14. The peptide-cargo complex of claim 6 wherein the cargo is glutathione.

15. The peptide-cargo complex of claim 6 wherein the peptide has the amino acid sequence TLPSPLALLTVH (SEQ ID NO:59).

16. The peptide-cargo complex of claim 6 wherein the peptide has the amino acid sequence SVSVGMKPSPRP (SEQ ID NO:86).

17. The peptide-cargo complex of claim 6 wherein the peptide is biotinylated and the cargo is avidin-labeled.

18. An expression cassette comprising a DNA encoding a fusion protein comprising a leader sequence, a protein of interest and an internalizing peptide having an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

19. The expression cassette of claim 18 further comprising expression control sequences operatively linked to said DNA.

20. A transfer vector comprising the expression cassette of claim 19.

21. The expression cassette of claim 18, wherein said leader sequences are selected from the group consisting of II-1ra, PTH, VP-22 and related sequences.

22. The expression cassette of claim 18 wherein the protein of interest is selected from the group consisting of apoptotic proteins, anti-apoptotic proteins, cell cycle regulatory proteins, transcription factors, suicide gene products, viral or tumor antigens, and cell proliferation factors.

23. The expression cassette of claim 18, wherein the encoded fusion protein comprises an amino acid sequence which facilitates removal of leader sequences therefrom and wherein said leaderless fusion protein comprises an internalizing peptide and a protein of interest.

24. The expression cassette of claim 18 wherein said fusion protein encoded thereby is produced and secreted from a cell and subsequently internalized into surrounding cells.

25. A method for inducing synovial cell death comprising administering a peptide-cargo complex to said synovial cell, wherein the peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID

ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

26. A method for inducing apoptosis in a tumor cell comprising administering a peptide-cargo complex to said tumor cell, wherein the peptide has the amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

27. A method for reducing white blood cells in arthritic joints comprising administering a peptide-cargo complex to said white blood cells, wherein the peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

28. A method for inhibiting apoptosis in an islet cell comprising administering a peptide-cargo complex to said islet cell, wherein the peptide has an amino acid sequence selected from the group consisting of KRIIQRILSRNS (SEQ ID NO: 1), KRIHPRLTRSIR (SEQ ID NO:2), PPRLRKRRQLNM (SEQ ID NO:3), PIRRRKKLRRLK (SEQ ID NO:4), RRQRRTSKLMKR(SEQ ID NO:5), MHKRPTTPSRKM (SEQ ID NO:6), RQRSRRRPLNIR (SEQ ID NO:7), RIRMIQNLIKKT (SEQ ID NO:8), SRRKRQRSNMRI (SEQ ID NO:9), QRIRKSKISRTL (SEQ ID NO:10), PSKRLLHNNLRR (SEQ ID NO: 11), HRHIRRQSLIML (SEQ ID NO: 12), PQNRLQIRRHSK (SEQ ID NO: 13), PPHNRIQRRLNM (SEQ ID NO:14), SMLKRNHSTSNR (SEQ ID NO: 15), GSRHPSLIIPRQ (SEQ ID NO: 16), SPMQKTMNLPPM (SEQ ID NO:17), NKRILIRIMTRP (SEQ ID NO:18), HGWZIHGLLHRA (SEQ ID NO:25), AVPAKKRZKSV (SEQ ID NO:26), PNTRVRPDVSF (SEQ ID NO:27), LTRNYEAWVPTP(SEQ ID NO:28), SAETVESCLAKSH (SEQ ID NO:29), YSHIATLPFTPT (SEQ ID NO:30), SYIQRTPSTTLP (SEQ ID NO:31), AVPAENALNNPF (SEQ ID NO:32), SFHQFARATLAS(SEQ ID NO:33), QSPTDFTFPNPL (SEQ ID NO:34), HFAAWGGWSLVH (SEQ ID NO:35), HIQLSPFSQSWR (SEQ ID NO:36), LTMPSDLQPVLW (SEQ ID NO:37), FQPYDHPAEVSY (SEQ ID NO:38), FDPFFWKYSPRD (SEQ ID NO:39), FAPWDTASFMLG (SEQ ID NO:40), FTYKNFFWLPEL (SEQ ID NO:41), SATGAPWKMWVR (SEQ ID NO:42), SLGWMLPFSPPF (SEQ ID NO:43), SHAFTWPTYLQL (SEQ ID NO:44), SHNWLPLWPLRP (SEQ ID NO:45), SWLPYPWHVPSS (SEQ ID NO:46), SWWTPWHVHSES (SEQ ID NO:47), SWAQHLSLPPVL (SEQ ID NO:48), SSSIFPPWLSFF (SEQ ID NO:49), LNVPPSWFLSQR(SEQ ID NO:50), LDITPFLSLTLP (SEQ ID NO:51), LPHPVLHMGPLR (SEQ ID NO:52), VSKQPYYMWNGN (SEQ ID NO:53), NYTTYKSHFQDR (SEQ ID NO:54), AIPNNQLGFPFK (SEQ ID NO:55), NIENSTLATPLS (SEQ ID NO:56), YPYDANHTRSPT (SEQ ID NO:57), DPATNPGPHFPR (SEQ ID NO:58), TLPSPLALLTVH (SEQ ID NO:59), HPGSPFPPPEHRP (SEQ ID NO:60), TSHTDAPPARSP (SEQ ID NO:61), MTPSSLSTLPWP (SEQ ID NO:62), VLGQSGYLMPMR (SEQ ID NO:63), QPIIITSPYLPS (SEQ ID NO:64), TPKTMTQTYDFS (SEQ ID NO:65), NSGTMQSASRAT (SEQ ID NO:66), QAASRVENYMHR (SEQ ID NO:67), HQHKPPPLTNNW (SEQ ID NO:68), SNPWDSLLSVST (SED ID NO:69), KTIEAHPPYYAS (SEQ ID NO:70), EPDNWSLDFPRR(SEQ ID NO:71), HQHKPPPLTNNW (SEQ ID NO:72), GVVGKLGQRRTKKQRRQKK (SEQ ID NO:73), GRRTKKQRRQKKPPRYMILGLLALAAVCSAA (SEQ ID NO:74), GRRTKKQRRQKKPP (SEQ ID NO:75), MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

29. The method of claim 26, wherein the tumor cell is a prostate tumor cell.

30. A method for delivering anti-oxidant and anti-inflammatory agents to lung epithelial cells comprising administering a peptide-cargo complex to said lung epithelial cells, wherein the peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

31. The method of claim 25, 26, 27, 28, or 29 wherein the cargo is an apoptotic protein.

32. The method of claim 31 wherein the apoptotic protein is selected from the group consisting of p53, caspase-3, HSV thymidine kinase and an antimicrobial peptide.

33. The method of claim 32 wherein the antimicrobial peptide has an amino acid sequence selected from the group consisting of KLAKLAK (SEQ ID NO:22) and KLAKLAKKLAKLAK (SEQ ID NO:23).

34. The method of claim 30, wherein the anti-inflammatory agent is selected from the group consisting of NF-κB and CFTR peptides.

35. The method of claim 30, wherein the anti-oxidant is selected from the group consisting of superoxide dismutase (SOD) and manganese superoxide dismutase (MnSOD).

36. A method of internalization into a peptide-cargo complex into a cell, comprising administering to said cell an amount of said peptide-cargo complex and an agent which facilitates internalization.

37. The method of claim 53, wherein the agent is selected from the group consisting of dextran sulfate, heparan sulfate or protamine sulfate.

38. A method for internalizing a GST-fusion protein into a cell comprising administering to said cell a peptide-cargo complex and a GST fusion protein wherein the peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

39. The method of claim 38 wherein the cargo is glutathione.

40. A kit for internalizing a GST-fusion protein into a cell comprising a peptide-cargo complex wherein the peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

41. The kit according to claim 40 wherein the cargo is glutathione.

42. An immunogen comprising a peptide-cargo complex wherein said peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID

ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

43. The immunogen of claim 42 wherein the peptide has an amino acid sequence RRQRRTSKLMKR (SEQ ID NO:5).

44. The immunogen of claim 42 wherein the peptide has an amino acid sequence GVVGKLGQRRTKKQRRQKK (SEQ ID NO:73).

45. The immunogen of claim 42 wherein the cargo is selected from the group consisting of a polynucleotide, a polypeptide, a protein, a virus, a modified virus, a viral vector, and a plasmid.

46. The immunogen of claim 42 wherein the cargo is an antigen.

47. The immunogen of claim 42 wherein the cargo is an **HIV** protein selected from the group consisting of Gag, Pol, Env, Tat, Nef, **Vpr**, Vpv, Rev.

48. A method for eliciting an immune response in a subject comprising administering to a target cell of said subject an immunogen comprising a peptide-cargo complex wherein said peptide has an amino acid sequence selected from the group consisting of MYRPPAANVDPW (SEQ ID NO:76), SSPPPDLTTRTP (SEQ ID NO:77), ATTQSTPPAFHL (SEQ ID NO:78), SDLPHVSSYWRG(SEQ ID NO:79), TTTQFMEIRQSA (SEQ ID NO:80), GKTWKASDEDWT (SEQ ID NO:81), DPARILGRIFL (SEQ ID NO:82), YNLQPTTSARPT (SEQ ID NO:83), SLKTPTTSHLSQ (SEQ ID NO:84), TFDLRNNTHRNP (SEQ ID NO:85), SVSVGMKPSPRP (SEQ ID NO:86), RRQRR (SEQ ID NO:97), RRQRRQRR (SEQ ID NO:98), RRQRRQRRQRR (SEQ ID NO:99).

49. The method of claim 48 wherein the target cell is a mucosal cell.

50. The method of claim 49 wherein the mucosal cell is a cervical mucosal cell.

L16 ANSWER 17 OF 26 USPATFULL on STN
2003:23301 **VPR**-DRIVEN DNA OR RNA CONSTRUCT AND THERAPEUTIC USES THEREOF.
ALFIERI, CAROLINE, DORION, CANADA
TANNER, JEROME, DORION, CANADA
ROUX, PHILIPPE, MONTREAL, CANADA
US 2003017137 A1 20030123
APPLICATION: US 1998-120286 A1 19980722 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a DNA or RNA construct capable of expression of IL-2 in a warm-blooded animal or biological preparation, the recombinant DNA or RNA construct comprising a) a **Vpr** activated promoter; b) a transcribable DNA segment coding for IL-2 and; c) a secretory DNA encoding for a signal peptide functional in mammary cells and operably linked between the promoter and the DNA segment to facilitate secretion of the IL-2. The present invention also relates to a method for increasing the immune response of a warm-blooded animal or biological preparation. There is also described methods for inhibiting or stimulating expression of IL-8 of a warm-blooded animal or a biological preparation.
CLM What is claimed is:
1. A DNA or RNA construct capable of expression of IL-2 in a warm-blooded animal or biological preparation, said recombinant DNA or RNA construct comprising: a) a **Vpr** activated promoter; b) a transcribable DNA segment coding for IL-2 and; c) a secretory DNA encoding for a signal peptide functional in mammary cells and operably linked between said promoter and said DNA segment to facilitate secretion of said IL-2.

2. The DNA or RNA construct of claim 1 wherein said construct is

recombinant.

3. The DNA or RNA construct of claim 2 wherein the promoter is NFκB, NF-IL-6, NFκB/NF-IL-6 or comprises the bases corresponding to -94 to -72 of the NFκB/NF-IL-6 enhancer sequence.

4. The DNA or RNA construct of claim 2 wherein the promoter is selected from the group consisting of: a) NFκB recognition sequence: AGGAAATTCCA (SEQ ID NO:1); b) NF-IL-6 recognition sequence: CAGTTGCAAATCGTG (SEQ ID NO:2); c) NF-IL-6/NFκB recognition sequence: AGTTGCAAATCGTGGAATTTCCTGAA (SEQ ID NO:3); and d) -94 to -72 of the IL-8 core enhancer element NF-IL-6/NFκB recognition sequence: CAGTTGCAAATCGTGGAATTTCC (SEQ ID NO:4).

5. The DNA or RNA construct as defined in claim 4 wherein said transcribable IL-2 has the following sequence:

CCCCATAATA TTTTTCCAGA ATTAACAGTA TAAATTGCAT CTCTTGTTCA AGAGTTCCCT
60 (SEQ ID NO:5)

ATCACTCTCT TTAATCACTA CTCACAGTAA CCTCAACTCC TGCCACAATG TACAGGATGC
120

AACTCCTGTC TTGCATTGCA CTAAGTCTTG CACTTGTCAC AAACAGTGCA CCTACTTCAA
180

GTTCTACAAA GAAAACACAG CTACAACTGG AGCATTTACT GCTGGATTTA CAGATGATTT
240

TGAATGGAAT TAATAATTAC AAGAATCCCA AACTCACCAG CATGCTCACA TTTAAGTTTT
300

ACATGCCCAA GAAGGCCACA GAACTGAAAC ATCTTCAGTG TCTAGAAGAA GAACTCAAAC
360

CTCTGGAGGA AGTGCTAAAT TTAGCTCAAA GCAAAAACTT TCACTTAAGA CCCAGGGACT
420

TAATCAGCAA TATCAACGTA ATAGTTCTGG AACTAAAGGG ATCTGAAACA ACATTCATGT
480

GTGAATATGC TGATGAGACA GCAACCATTG TAGAATTTCT GAACAGATGG ATTACCTTTT
540

GTCAAAGCAT CATCTCAACA CTGACTTGAT AATTAAGTGC TTCCCACTTA AAACATATCA
600

GGCCTTCTAT TTATTTAAAT ATTTAAATTT TATATTTATT GTTGAATGTA TGGTTTGCTA
660

CCTATTGTAA CTATTATTCT TAATCTTAAA ACTATAAATA TGGATCTTTT ATGATTCTTT
720

TTGTAAGCCC TAGGGGCTCT AAAATGGTTT CACTTATTTA TCCCAAAATA TTTATTATTA
780

TGTTGAATGT TAAATATAGT ATCTATGTAG ATTGGTTAGT AAAACTATTT AATAAATTTG
840

ATAA
844

6. The DNA or RNA construct as defined in claim 5 wherein said transcribable further comprises a trancribable segment of IL-8.

transcribable IL-8 has the following sequence:

GAATTCAGTA ACCCAGGCAT TATTTTATCC TCAAGTCTTA GGTTGGTTGG AGAAAGATAA
60 (SEQ ID NO:6)

CAAAAAGAAA CATGATTGTG CAGAAACAGA CAAACCTTTT TGGAAAGCAT TTGAAAATGG
120

CATTCCCCCT CCACAGTGTG TTCACAGTGT GGGCAAATTC ACTGCTCTGT CGTACTTTCT
180

GAAAATGAAG AACTGTTACA CCAAGGTGAA TTATTTATAA ATTATGTACT TGCCCAGAAG
240

CGAACAGACT TTTACTATCA TAAGAACCCT TCCTTGGTGT GCTCTTTATC TACAGAATCC
300

AAGACCTTTC AAGAAAGGTC TTGGATTCTT TTCTTCAGGA CACTAGGACA TAAAGCCACC
360

TTTTTATGAT TTGTTGAAAT TTCTCACTCC ATCCCTTTTG CTGATGATCA TGGGTCCTCA
420

GAGGTCAGAC TTGGTGTCCT TGGATAAAGA GCATGAAGCA ACAGTGGCTG AACCAGAGTT
480

GGAACCCAGA TGCTCTTTCC ACTAAGCATA CAACTTTCCA TTAGATAACA CCTCCCTCCC
540

ACCCCAACCA AGCAGCTCCA GTGCACCACT TTCTGGAGCA TAAACATACC TTAACTTTAC
600

AACTTGAGTG GCCTTGAATA CTGTTCCTAT CTGGAATGTG CTGTTCTCTT TCATCTTCCT
660

CTATTGAAGC CCTCCTATTC CTCAATGCCT TGCTCCAACT GCCTTTGGAA GATTCTGCTC
720

TTATGCCTCC ACTGGAATTA ATGTCTTAGT ACCACTTGTC TATTCTGCTA TATAGTCAGT
780

CCTTACATTG CTTTCTTCTT CTGATAGACC AAACTCTTTA AGGACAAGTA CCTAGTCTTA
840

TCTATTTCTA GATCCCCCAC ATTACTCAGA AAGTTACTCC ATAAATGTTT GTGGAACTGA
900

TTTCTATGTG AAGACATGTG CCCCTTCACT CTGTTAACTA GCATTAGAAA AACAAATCTT
960

TTGAAAAGTT GTAGTATGCC CCTAAGAGCA GTAACAGTTC CTAGAAACTC TCTAAAATGC
1020

TTAGAAAAAG ATTTATTTTA AATTACCTCC CCAATAAAAT GATTGGCTGG CTTATCTTCA
1080

CCATCATGAT AGCATCTGTA ATTAACTGAA AAAAAATAAT TATGCCATTA AAAGAAAATC
1140

ATCCATGATC TTGTTCTAAC ACCTGCCACT CTAGTACTAT ATCTGTCACA TGGTCTATGA
1200

TAAAGTTATC TAGAAATAAA AAAGCATACA ATTGATAATT CACCAAATTG TGGAGCTTCA
1260

1320

CTCCGTATTT GATAAGGAAC AAATAGGAAG TGTGATGACT CAGGTTTGCC CTGAGGGGAT
1380

GGGCCATCAG TTGCAAATCG TGGAATTTCC TCTGACATAA TGAAAAGATG AGGGTGCATA
1440

AGTTCTCTAG TAGGGTGATG ATATAAAAAG CCACCGGAGC ACTCCATAAG GCACAAACTT
1500

TCAGAGACAG CAGAGCACAC AAGCTTCTAG GACAAGAGCC AGGAAGAAAC CACCGGAAGG
1560

AACCATTCTC ACTGTGTGTA AACATG
1586

8. The DNA or RNA construct as defined in claim 7 wherein the signal peptide is an IL-2 signal peptide or an analogue or derivative thereof.

9. The DNA or RNA construct as defined in claim 8 wherein the IL-2 signal peptide has the following sequence:

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
(SEQ ID NO:7)
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser
20 25

10. A method for increasing the immune response of a warm-blooded animal or biological preparation comprising the steps of: a) introducing a DNA or RNA construct as defined in claim 1 in stem cells, antigen presenting cells or immune cell leukocytes, fibroblasts and epithelial cells, of the warm-blooded animal or biological preparation to obtain a transfected cell populations; and b) administering a pharmaceutically effective amount of said transfected cell populations to the warm-blooded animal or biological preparation.

11. The method of claim 10 wherein said warm-blooded animal is an immunocompromised patient.

12. A method for inhibiting expression of IL-8 of a warm-blooded animal or a biological preparation comprising the step of administering a pharmaceutically effective amount of a **Vpr** inhibitor.

13. The method of claim 12 wherein said warm-blooded animal is an immunocompromised patient.

14. The method of claim 13 wherein the **Vpr** inhibitor is an anti-**Vpr antibody**.

15. The method of claim 14 wherein the anti-**Vpr antibody** is a monoclonal **antibody**.

16. A method for stimulating IL-8 expression in a mammal in need to, said method comprising the step of administering a pharmaceutically effective amount of a pharmaceutically acceptable formulation comprising a **Vpr** protein to said mammal.

17. A method for determining the interaction between **Vpr** and other proteins, said method comprises the steps of: a) co-precipitation of **Vpr** and associated cellular proteins using anti-**Vpr antibody** followed by protein gel electrophoresis; b) development of a yeast two hybrid system in which a **Vpr**-Gal4 construct is introduced into yeast

insensitive colonies; and c) constriction of **Vpr** deletion mutants to identify both association of cellular proteins with **Vpr** or **Vpr** subdomains.

L16 ANSWER 18 OF 26 USPATFULL on STN
2002:322428 Compositions and methods for evaluating viral receptor/co-receptor usage and inhibitors of virus entry using recombinant virus assays.
Petropoulos, Christos J., Half Moon Bay, CA, UNITED STATES
Parkin, Neil T., Belmont, CA, UNITED STATES
Whitcomb, Jeannette M., San Mateo, CA, UNITED STATES
Huang, Wei, Foster City, CA, UNITED STATES
US 2002182592 A1 20021205
APPLICATION: US 2001-874475 A1 20010604 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a method for identifying whether a compound inhibits entry of a virus into a cell which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the compound, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the compound indicates that the compound inhibits entry of the virus into the second cell.

CLM What is claimed is:

1. A method for identifying whether a compound inhibits entry of a virus into a cell which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the compound, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the compound indicates that the compound inhibits entry of the virus into the second cell.

2. The method of claim 1, wherein the indicator nucleic acid comprises an indicator gene.

3. The method of claim 2, wherein the indicator gene is a luciferase gene.

4. The method of claim 1, wherein the cell surface receptor is CD4.

5. The method of claim 1, wherein the cell surface receptor is a chemokine receptor.

6. The method of claim 1, wherein the cell surface receptor is CXCR4 or

7. The method of claim 1, wherein the patient is infected with the **HIV**-1 virus.

8. The method of claim 1, wherein the nucleic acid of step (a) comprises DNA encoding gp120 and gp41.

9. The method of claim 1, wherein the viral expression vector comprises **HIV** nucleic acid.

10. The method of claim 9, wherein the viral expression vector comprises an **HIV** gag-pol gene.

11. The method of claim 9, wherein the viral expression vector comprises DNA encoding vif, **vpr**, tat, rev, vpu, and nef.

12. The method of claim 1, wherein the first cell is a mammalian cell.

13. The method of claim 12, wherein the mammalian cell is a human cell.

14. The method of claim 13, wherein the human cell is a human embryonic kidney cell.

15. The method of claim 14, wherein the human embryonic kidney cell is a 293 cell.

16. The method of claim 1, wherein the second cell is a human T cell.

17. The method of claim 1, wherein the second cell is a human T cell leukemia cell line.

18. The method of claim 1, wherein the second cell is a peripheral blood mononuclear cell.

19. The method of claim 1, wherein the second cell is an astroglioma cell.

20. The method of claim 19, wherein the astroglioma cell is a U87 cell.

21. The method of claim 1, wherein the second cell is a human osteosarcoma cell.

22. The method of claim 2, wherein the human osteosarcoma cell is an HT4 cell.

23. The method of claim 1, wherein the compound binds to the cell surface receptor.

24. The method of claim 1, wherein the compound is a ligand of the cell surface receptor.

25. The method of claim 23, wherein the compound comprises an **antibody**.

26. The method of claim 1, wherein the compound inhibits membrane fusion.

27. The method of claim 1, wherein the compound is a peptide, a peptidomimetic, an organic molecule, or a synthetic compound.

28. The method of claim 1, wherein the compound binds the viral envelope protein.

29. A method for making a composition which comprises admixing the compound identified by claim 1 with a carrier.

30. The method of claim 29, wherein the carrier is saline, polyethylene glycol, a buffer solution, a starch, or an organic solvent.

31. A method for identifying a cell surface receptor which is bound by a virus upon infection of a cell by the virus which comprises: (a) obtaining viral particles which comprise (i) a viral nucleic acid and (ii) an indicator nucleic acid which produces a detectable signal; (b) contacting a cell which expresses a cell surface receptor with the viral particles from step (a); and (c) measuring the amount of detectable signal produced within the cell, wherein production of the signal indicates the cell surface receptor expressed by the cell is bound by the virus, thereby identifying the cell surface receptor as being bound by the virus upon infection of the cell.

32. A method for identifying whether an **antibody** inhibits entry of a virus into a cell which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the **antibody**, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the **antibody** indicates that the **antibody** inhibits entry of the virus into the second cell.

33. A method for determining susceptibility of a virus to a compound which inhibits viral cell entry which comprises: (a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus; (b) co-transfecting into a first cell (i) the nucleic acid of step (a), and (ii) a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces a detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient; (c) contacting the viral particles produced in step (b) with a second cell in the presence of the compound, wherein the second cell expresses a cell surface receptor to which the virus binds; (d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and (e) comparing the amount of signal measured in step (d) with the amount of signal produced in the absence of the compound, wherein a reduced amount of signal measured in the presence of the compound indicates that the virus is susceptible to the compound.

34. A method for determining resistance of a virus to a compound which inhibits viral entry into a cell which comprises: (a) determining susceptibility of a virus to a compound according to the method of claim 33, wherein a nucleic acid encoding a viral envelope protein is obtained from a patient at a first time; (b) determining susceptibility of the virus to the compound according to the method of claim 33, wherein the nucleic acid encoding the viral envelope protein is obtained from the patient at a later second time; and (c) comparing the susceptibilities determined in steps (a) and (b), wherein a decrease in susceptibility at the later second time indicates resistance of the virus to the compound.

35. A method for identifying a mutation in a virus that confers resistance to a compound that inhibits viral entry into a cell which comprises: (a) determining the nucleic acid sequence or the amino acid sequence of the virus prior to any treatment of the virus with the compound; (b) obtaining a virus resistant to the compound; (c)

determining the nucleic acid sequence of the amino acid sequence of the resistant virus from step (b); and (d) comparing the nucleic acid sequence or the amino acid sequences of steps (a) and (c), respectively, so as to identify the mutation in the virus that confers resistance to the compound.

36. The method of claim 35, wherein the virus obtained in step (b) is the virus of step (a) grown in the presence of the compound until resistance is developed.

37. The method of claim 35, wherein the virus obtained in step (b) is isolated from a patient which has been undergoing treatment with the compound.

L16 ANSWER 19 OF 26 USPATFULL on STN
2002:294293 Mammalian cytokines; related reagents and methods.
Hedrick, Joseph A., Plainsboro, NJ, UNITED STATES
Sana, Theodore R., East Palo Alto, CA, UNITED STATES
Bazan, J. Fernando, Menlo Park, CA, UNITED STATES
Kastelein, Robert A., Redwood City, CA, UNITED STATES
US 2002164332 A1 20021107
APPLICATION: US 2001-770528 A1 20010125 (9)
PRIORITY: US 1997-44165P 19970421 (60)
US 1997-55111P 19970806 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Nucleic acids encoding mammalian, e.g., rodent, IL-1δ, IL-1ε, purified IL-1δ and IL-1ε proteins and fragments thereof. **Antibodies**, both polyclonal and monoclonal, are also provided. Methods of using the compositions for both diagnostic and therapeutic utilities are provided.

CLM What is claimed is:
1. An isolated or recombinant polypeptide: A) that: a) specifically binds polyclonal **antibodies** generated against a 12 consecutive amino acid segment of SEQ ID NO: 2; and b) comprises at least one sequence selected from the following group (see SEQ ID NO: 2):

LeuCysPheArgMetLysAsp; ValLeuTyrLeuHisAsn;

GlnLeuLeuAlaGly; IleSerValValProAsn;

SerProValIleLeuGlyVal; GlnCysLeuSerCysGlyThr;

ProIleLeuLysLeuGlu; PheTyrArgArgAspMetGly;

LeuThrSerSerPheGluSer; PheLeuCysThrSer;

GlnProValArgLeuThr; PheTyrPheGlnGln;

ArgAlaLeuAspAlaSerLeu; and GlyLeuHisAlaGluLysVal;

B) that: a) specifically binds polyclonal **antibodies** generated against a 12 consecutive amino acid segment of SEQ ID NO: 6; and b) comprises at least one sequence selected from the following group (see SEQ ID NO: 6):

SerLeuArgHisValGlnAsp; ValTrpIleLeuGlnAsn;

IleLeuThrAlaVal; IleThrLeuLeuProCys;

AspProThrTyrMetGlyVal; SerCysLeuPheCysThrLys;

ProValLeuGlnLeuGly; PheTyrHisLysLysSerGly;

ThrThrSerThrPheGluSer; PheIleAlaValCys;

CysProLeuIleLeuThr; PheGluMetIleVal;

GlnAspLeuSer;
ValProArgLysGluGlnThrVal;

SerLysGlySerCysPro; ArgAlaAlaSer;

ProCysGlnTyrLeuAspThrLeuGlu; and SerGlyThrThr; or

C) that: a) specifically binds polyclonal **antibodies** generated against a 12 consecutive amino acid segment of SEQ ID NO: 13 or 15; and b) comprises at least one sequence selected from the following group (see SEQ ID NO: 13 or 15):

ITGTIND; VWTLQG; NLVAV; VAVITC; DPIYLGI; MCLYCEK;

PTLQLK; FYPAKTG; RTSTLES; FIASS; QPIILT; FELNI;

SMCK; NDLN; **VPR**(R/S)TSVT; VPRSDSVT; TCKYPEALE;

TGRT; SKRDQP; or SKGDQP.

2. The polypeptide of claim 1: a) wherein said polypeptide comprises a plurality of said sequences selected from said group in section b) of part 1A; b) wherein said polypeptide comprises a plurality of said sequences selected from said group in section b) of part 1B; c) wherein said polypeptide comprises a plurality of said sequences selected from said group in section b) of part 1C; or d) which specifically binds to polyclonal **antibodies** generated against an immunogen selected from the group consisting of: i) the polypeptide of SEQ ID NO: 2; ii) the polypeptide of SEQ ID NO: 6; iii) the polypeptide of SEQ ID NO: 13; and. iv) the polypeptide of SEQ ID NO: 15.

3. The polypeptide of: A) claim 1A, wherein said 12 consecutive amino acid segment is selected from (see SEQ ID NO: 2):

LeuCysPheArgMetLysAspSerAlaLeuLysValLeuTyrLeuHis-

`````AsnAsn;

IleSerValValProAsnArgAlaLeuAspAlaSerLeuSerProVal-

`````IleLeuGlyValGln;

SerProValIleLeuGlyValGlnGlyGlySerGlnCys;

ProIleLeuLysLeuGluProValAsnIleMetGluLeu;

ThrSerSerPheGluSerAlaAlaTyrProGlyTrpPhe;

PheLeuCysThrSerProGluAlaAspGlnProVal;

ThrGlnIleProGluAspProAlaTrpAspAlaProIle; or

ThrSerSerPheGluSerAlaAlaTyrProGlyTrpPhe;

B) claim 1B, wherein said 12 consecutive amino acid segment is selected from (see SEQ ID NO: 6):

ArgAlaAlaSerProSerLeuArgHisValGlnAspLeu;

SerSerArgValTrpIleLeuGlnAsnAsnIleLeu;

ProValThrIleThrLeuLeuProCysGlnTyrLeu;

PheCysThrLysAspGlyGluGlnProValLeuGlnLeu;

ThrSerThrPheGluSerAlaAlaPheProGlyTrpPhe; and

CysSerLysGlySerCysProLeuIleLeuThrGln; or

C) claim 1C, wherein said 12 consecutive amino acid segment is selected from (see SEQ ID NO: 13 or 15):

SMCKPITGTINDL;

NQQVWTLQGQNL;

PVTVAVITCKYP;

GIQNPEMCLYCE;

YCEKVGEQPTLQL;

TSTLESVAFPDWF;

SKGDQPIILTSE;

SKRDQPIILTSE; and

GKSYNTAFELNIND.

3. The polypeptide of claim 2, wherein said polypeptide: i) is a mature protein; ii) lacks a post-translational modification; iii) is from a rodent, including a mouse; iv) is from a primate, including a human; v) is a natural allelic variant of IL-1δ or IL-1ε; vi) has a length at least 30 amino acids; vii) exhibits at least two non-overlapping epitopes that are specific for a rodent IL-1δ; viii) exhibits a sequence identity over a length of at least about 20 amino acids to SEQ ID NO: 2; ix) exhibits at least two non-overlapping epitopes which are specific for a rodent or primate IL-1ε; x) exhibits a sequence identity over a length of at least about 20 amino acids to SEQ ID NO: 6 or 15; xi) is glycosylated; xii) has a molecular weight of at least 10 kD with natural glycosylation; xiii) is a synthetic polypeptide; xiv) is attached to a solid substrate; xv) is conjugated to another chemical moiety; xvi) is a 5-fold or less substitution from natural sequence; or xvii) is a deletion or insertion variant from a natural sequence.

4. A soluble polypeptide comprising: a) a sterile polypeptide of claim 2; b) said sterile polypeptide of claim 2 and a carrier, wherein said carrier is: i) an aqueous compound, including water, saline, and/or buffer; and/or ii) formulated for oral, rectal, nasal, topical, or parenteral administration.

5. A fusion protein having a polypeptide sequence of claim 2 and further comprising: a) a mature protein of claim 2; b) a detection or purification tag, including a FLAG, His6, or Ig sequence; or c) sequence of another cytokine or chemokine.

6. A kit comprising a polypeptide of claim 2, and: a) a compartment comprising said protein or polypeptide; and/or b) instructions for use or disposal of reagents in said kit.

7. A binding compound comprising an antigen binding site from an **antibody**, which specifically binds to a mature polypeptide from: a) SEQ ID NO: 2; b) SEQ ID NO: 6; c) SEQ ID NO: 13; or d) SEQ ID NO: 15.

8. The binding compound of claim 7, wherein: a) said binding compound

is an Fv, Fab, or Fab2 fragment; b) said binding compound is conjugated to another chemical moiety; or c) said **antibody**: i) is raised against a polypeptide comprising a 12 consecutive amino acid segment of SEQ ID NO: 2, 6, 13, or 15; ii) is raised against a mature IL-1ε; iii) is raised to a purified rodent IL-1δ or rodent or primate IL-1ε; iv) is immunoselected; v) is a polyclonal **antibody**; vi) binds to a denatured IL-1δ or IL-1ε; vii) exhibits a Kd to antigen of at least 30 μM; viii) is attached to a solid substrate, including a bead or plastic membrane; ix) is in a sterile composition; or x) is detectably labeled, including a radioactive or fluorescent label.

9. A kit comprising said binding compound of claim 7, and: a) a compartment comprising said binding compound; and/or b) instructions for use or disposal of reagents in said kit.

10. A composition comprising: a) a sterile binding compound of claim 7, or b) said binding compound of claim 7 and a carrier, wherein said carrier is: i) an aqueous compound, including water, saline, and/or buffer; and/or ii) formulated for oral, rectal, nasal, topical, or parenteral administration.

11. An isolated or recombinant nucleic acid encoding a polypeptide of claim 2, wherein: a) said polypeptide of claim 2 is IL-1δ or IL-1ε from a mammal; or b) said nucleic acid: i) comprises the mature coding sequence of SEQ ID NO: 1, 3, 12, or 14; ii) encodes an antigenic peptide sequence of SEQ ID NO: 2, or SEQ ID NO: 6, 13, or 15; iii) encodes a plurality of antigenic peptide sequences of SEQ ID NO: 2, or SEQ ID NO: 6, 13, or 15; iv) exhibits identity to a natural cDNA encoding said segment; v) is an expression vector; vi) further comprises an origin of replication; vii) is from a natural source; viii) comprises a detectable label; ix) comprises synthetic nucleotide sequence; x) is less than 6 kb, preferably less than 3 kb; xi) is from a rodent or primate; xii) comprises a natural full length coding sequence; xiii) is a hybridization probe for a gene encoding said IL-1δ or IL-1ε; xiv) is a PCR primer, PCR product, or mutagenesis primer; or xv) encodes an IL-1δ or an IL-1ε protein.

12. A cell, transformed with said nucleic acid of claim 10.

13. The cell of claim 12, wherein said cell is: a) a prokaryotic cell; b) a eukaryotic cell; c) a bacterial cell; d) a yeast cell; e) an insect cell; f) a mammalian cell; g) a murine cell; h) a primate cell; or i) a human cell.

14. A kit comprising said nucleic acid of claim 11, and: a) a compartment comprising said nucleic acid; b) a compartment further comprising a mammalian IL-1δ or IL-1ε protein or polypeptide; and/or c) instructions for use or disposal of reagents in said kit.

15. An isolated or recombinant nucleic acid that a) hybridizes under wash conditions of 40° C. and less than 1M salt to SEQ ID NO: 1; b) hybridizes under wash conditions of 40° C. and less than 1 M salt to SEQ ID NO: 3, 5, 12 or 14.

16. The nucleic acid of claim 15, wherein: a) said wash condition is at 50° C. and/or 500 mM salt; and b) exhibits identity over at least 20 nucleotides to SEQ ID NO: 1, 3, 5, 12, or 14.

17. The nucleic acid of claim 16, wherein: a) a wash condition is at 65° C. and/or 150 mM salt; or b) exhibits identity over at least 50 nucleotides to SEQ ID NO: 1, 3, 5, 12, or 14.

comprising contacting said cell with an agonist or antagonist of a mammalian IL-1δ or IL-1ε polypeptide of claims 1.

19. The method of claim 18, wherein: a) said contacting is in combination with an agonist or antagonist of IL-1α, IL-1RA, IL-1β, IL-1γ, IL-2, and/or IL-12; b) said contacting is with an antagonist, including binding composition comprising an **antibody** binding site which specifically binds an IL-1δ or IL-1ε; or c) said modulating is regulation of IFN-γ production.

20. A method of: A) making an antiserum comprising an **antibody** of claim 7, comprising immunizing a mammal with an immunogenic amount of: a) a rodent IL-1δ polypeptide; b) a peptide sequence comprising a 12 consecutive amino acid segment of SEQ ID NO: 2; c) a rodent or primate IL-1ε polypeptide; or d) a peptide sequence comprising a 12 consecutive amino acid segment of SEQ ID NO: 6, 13, or 15; thereby causing said antiserum to be produced; or B) producing an antigen:**antibody** complex, comprising contacting: a) a rodent IL-1δ protein or peptide with an **antibody** of claim 7; or b) a rodent or primate IL-1ε protein or peptide with an **antibody** of claim 7; thereby allowing said complex to form.

L16 ANSWER 20 OF 26 USPATFULL on STN
2002:227883 Method for analysing **human immunodeficiency virus** (**HIV**) phenotypic characteristics.
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US 2002123036 A1 20020905
APPLICATION: US 2001-817135 A1 20010327 (9)
PRIORITY: FR 2000-14495 20001110
FR 2001-3970 20010323
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for analyzing the phenotypic characteristics shown by certain virus strains, particularly human immnunodeficiency viruses, involving the construction of a recombinant virus obtained by homologous recombination.

The present invention also relates a kit comprising the primers, vectors, cell hosts, products and reagents required to carry out PCR amplification, and the products and reagents used to detect a marker, for the implementation of the method according to the invention.
CLM What is claimed is:
1. Method for analysing a phenotypic characteristic of **HIV** viruses present in a biological specimen from a patient, said phenotypic characteristic resulting from one or more mutations of the viral genome liable to influence the viral infection, characterised in that it comprises: a) the extraction of the nucleic acids contained in said biological specimen, b) at least one PCR amplification of a segment of the nucleic acids from step (a), each with a pair of primers bordering a nucleic acid sequence of the viral genome liable to comprise at least one mutation, c) the preparation of a vector comprising the parts of an **HIV** virus genome required for viral replication except for the segment amplified in step (b) and, if applicable, except for the gene coding for envelope protein, d) the transfection of a first cell host with: the nucleic acids contained in step (b), the vector prepared in step (c), if applicable, a second vector comprising a gene coding for an envelope protein if the envelope gene is deleted from the vector prepared in step (c), to obtain a chimeric virus by homologous recombination, e) the

of viral particles during a single replication cycle, f) the injection by the viral particles obtained in step (e) of at least one second cell host liable to be infected by an **HIV** virues or on **HIV** pseudotype virus and comprising, if applicable, a marker gene that can only be activated following viral infection, g) the detection and/or quantification of the marker expressed in step (f) in order to detect at least one characteristic of the **HIV** viruses present in the biological specimen.

2. Analytical method according to claim 1 characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence comprising all or part of a viral genomc region selected from: gag, pol, protease, reverse transcriptase, RNAsc H, integrase, vif, **vpr**, tat, rcv, vpu, env, nef, cis-active sequences, LTR, dimerisation sequences, splicing regulating sequences or Rev response element (RRE).

3. Analytical method according to any of claim 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence coding for a part of the gag protein of the human immune deficiency virus and a nucleic acid sequence coding for protease, liable to comprise at least one mutation in the gene coding for protease and, in that the vector from step (c) is constructed from an **HIV** virus genome in which all or part of the gene coding for protease is deleted.

4. Analytical method according to claims 1 to 3 characterised in that the amplification in step (b) comprising at least one mutation in the gene coding for protease is performed with a pair of primers: Fit A-: (5' TCA CCT AGA ACT TTA AAT GC 3') (SEQ ID No: 1) and Pro A-: (5' GGC AAA TAC TGG AGT ATT GTA TG3' 3' (SEQ ID No: 2), followed by a second amplification with a pair of primers: Fit B: (5' AGA ACT TTA AAT GCA TGG GT 3') (SEQ ID No: 3) and Pro B-: (5' GGA GTA TTG TAT GGA TTT TCA GG 3') (SEQ ID No: 4), to obtain a DNA segment with 1460 base pairs, ranging from the residues 3950 and 5410 inclusive, and in that the vector from step (c) is a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 protease ranging from the residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a single M1uI restriction site.

5. Analytical method according to claim 1 or 2 characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for reverse transcriptase, and the transfection in step (c) is carried out with a first vector constructed from an **HIV** virus genome in which all or part of the gene coding for reverse transcriptase is deleted.

6. Analytical method according to claim 1, 2 or 5, characterised in that the amplification in step (b) is performed with a pair of primers: MJ3 (5' AGT AGG ACC TAC ACC TGT CA 3') (SEQ ID No: 5) and RT-EXT (5' TTC CCA ATG CAT ATT GTG AG 3') (SEQ ID No: 6), followed by a second amplification step with a pair of primers: A35 (5' TTG GTT GCA TAA ATT TTC CCA TTA GTC CTA TT 3') (SEQ ID No: 7) and RT-IN (5' TTC CCA ATG CAT ATT GTG AG 3') (SEQ ID No): 8) to obtain a DNA sequence with 1530 base pairs ranging beyond codon 93 of the region coding for protease and beyond codon 503 of the region coding for polymerase (POL) and in that the vector from step (c) is a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 reverse transcriptase ranging from the residues 2618 to 2872 inclusive, and comprising a single M1uI restriction site.

7. Analytical method according to claim 1, 2, 5 or 6 consisting of determining the susceptibility of an **HIV** virus to a reverse transcriptase inhibiting compound, characterised in that said reverse

transcriptase inhibiting compound is added or not, possibly at different concentrations, to the second cell host, before the infection of said host by the viral particles obtained in step (e), and in that step (v) comprises the comparison of the expression of the marker gene with and without reverse transcriptase inhibiting compound.

8. Analytical method according to claim 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for integrase, and the vector in step (c) is a retroviral vector in which all or part of the gene coding for intergrase is deleted.

9. Analytical method according to claim 1, 2 or 8, characterised in that the amplification in step (b) is performed with the pair of primers: INT B+ -5'GTTACTAATAGAGGAAGACAA3'(SEQ ID) No: 9) and INT B- 5'TTTTGGTGTTATTAATGCT3' (SEQ ID No: 10), followed by a second amplification step with the pair of primers: INT V+ 5'CACCCTAACTGACACAACAA3' (SEQ ID No: 11) and INT V- 5'AAGGCCTTTCTTATAGCAGA3' (SEQ ID No: 12), to obtain a DNA segment with 1460 base pairs ranging from residues 3950 to 5410 inclusive and in that the vector from step (c) is a retroviral vector deleted from the entire region of the pol reading frame coding for **HIV**-1 integrase ranging from the residues 4228to 5093 inclusive and the region coding for the viral envelope between the positions 6343 and 7611 inclusive.

10. Analytical method according to claim 1, 2, 8 or 9 consisting of determining the susceptibility of an **HIV** virus to an integrase inhibiting compound, characterised in that said integrase inhibiting compound is added, possibly at different concentrations, during step (e), before step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without integrase inhibiting compound.

11. Analytical method according to claim 1 or 2, characterised in that the PCR amplification in step (b) is carried out with a pair of primers bordering a nucleic acid sequence liable to comprise at least one mutation in the gene coding for envelope protein, and in that the vector from step (c) is a retroviral vector constructed from an **HIV** virus genome in which all or part of the gene coding for envelope protein is deleted.

12. Analytical method according to claim 1, 2 or 11 characterised in that the vector from step (c) is a retroviral vector deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, the region of the **HIV**-1 genome forming the Rev response element (RRE).

13. Analytical method according to claim 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: FIN-A: 5'TCAAATATTACAGGGCTGCT3' (SEQ ID No: 13) and FIN-B: 5'TAGCTGAAGAGGCACAGG3' (SEQ ID No: 14) followed by a second amplification step, performed with the pair of primers: FIN-C: 5'CTATTAACAAGAGATGGTGG3' (SEQ ID No: 15) and FIN-D: 5'TCCACCTTCTTCTTCGATT3' (SEQ ID No: 16), to obtain a DNA segment with 965 base pairs ranging from the residues 7553 to 8517 inclusive and in that the vector in step (c) is a retroviral virus deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, and comprises a single Mul1 restriction site.

14. Analytical method according to claim 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: FuA: 5'AAGCAATGTATGCCCCTCCCAT3' (SEQ ID No: 23) and FuB: 5'GGTGGTAGCTGAAGAGGCACAGG3' (SEQ ID No: 24) followed by a second

amplification step, performed with the primers: FuD1: 5'ATATGAGGGACAATTGGAGAAGTGA3' (SEQ ID No: 25) and a mixture of the following primers: FuD1: 5'TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No: 26) FuD2: 5'TCTGTCTTGCTCTCCACCTTCTTCTT3' (SEQ ID No: 27), said mixture being preferently carried out in a ratio comprised between (10%:90%) and (90%:10%) more preferently between (60%:40%) and (40%:60%), to obtain a DNA segment with 805 base pairs ranging from the residues 7635 to 8440 inclusive and the vector in step c is a retroviral virus deleted from the entire region coding for the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 7745 to 8263 inclusive, and comprises a single MulI restriction site.

15. Analytical method according to claim 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: NEU-A: 5'TAGAAAGAGCAGAAGACAGTGGCAATCG3' (SEQ ID No: 17) and FIN-B: 5'TAGCTGAACAGGCACAGG3' (SEQ ID No: 14) followed by a second amplification step, performed with the pair of primers: NEU-C: 5'GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18) and FIN-D: 5'TCCACCTTCTTCTTCGATT3' (SEQ ID No: 16), to obtain a DNA segment with 2320 base pairs ranging from the residues 6197 to 8517 inclusive and in that the vector in step (c) is a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 6480 to 8263 inclusive, and comprises a single Mul1 restriction site.

16. Analytical method according to claim 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: NEU-A: 5'TAGAAAGAGCAGAAGACAGTGGCAATG3' (SEQ ID No: 17) and FuB: 5' GGTGGTAGCTGAAGAGGCACAGG3' (SEQ ID No: 24), followed by a second amplification step, performed with the pair of primers: NEU-C: 5'GTGGGTCACAGTCTATTATGGGG3' (SEQ ID No: 18) and a mixture of the following primers FuD1: 5'TCTGTCTCTCTCTCCACCTTCTTCTT3' (SEQ ID No: 26) and FuD2: 5'TCTGTCTTGCTCTCCACCTTCTTCTT3' (SEQ ID No: 27), said mixture being preferently carried out in a ratio comprised between (10%:90%) and (90%:10%) more preferently between (60%:40%) and (40%:60%), to obtain a DNA segment with 2118 base pairs ranging from the residues 6322 to 8440 inclusive and the vector in step c is a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the gp41 sub-unit of the **HIV**-1 envelope, ranging from the residues 6480 to 8263 inclusive, and comprises a single Mul1 restriction site.

17. Analytical method according to claim 1, 2, 11 or 12, characterised in that the amplification in step (b) is performed with a pair of primers: E00: 5'TAGAAAGAGCAGAAGACAGTGGCAATGA3' (SEQ ID No: 19) and ES8B: 5'CACTTCTCCAATTGTCCCTCA3' (SEQ ID No: 22), followed by a second amplification step, performed with the pair of primers: E20: 5'GGGCCACACATGCCTGTACCCACAG3' (SEQ ID No: 21) and E115: 5'AGAAAAATTCCCCTCCACAATTAA3' (SEQ ID No: 22), to obtain a DNA segment with 938 base pairs ranging from the residues 6426 to 7364 inclusive and in that the vector in step (c) is a retroviral vector deleted from the region, coding for the domains ranging from the loop V1 to the loop V3 of the **HIV**-1 envelope ranging from 6617 to 7250 inclusive and comprises a single NheI restriction site.

18. Analytical method according to claims 1, 2, 11 to 17 consisting of determining the susceptibility of an **HIV** virus to a fusion inhibiting compound targeting **HIV**-1 gp41 protein, characterised in that said fusion inhibiting compound is added, possibly at different concentrations, during the culture of the cell host obtained in step (e), before step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without fusion inhibiting compound targeting **HIV**-1 gp41.

19. Analytical method according to claim 1, 2, 11, 12 or 15 consisting

of determining the susceptibility of an **HIV** virus to a compound inhibiting the entry of said **HIV** virus into a target cell, characterised in that said entry inhibiting compound is added, possibly at different concentrations, to the cell host obtained in step (e) before the infection in step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without entry inhibiting compound.

20. Analytical method according to claim 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to the inhibitory action of **antibodies**, characterised in that said method is carried out, firstly without **antibodies** and, secondly, with the **antibody**, possibly at different concentrations, said **antibody** being present in step (e), and in that step (g) comprises the comparison of the expression of the marker gene with and without **antibodies**.

21. Analytical method according to claim 1, 2, 11, 12 or 15 consisting of determining the tropism of an **HIV** virus for a cell receptor, characterised in that the infection in step (f) with the viral particles obtained in step (e) is performed on two separate cell hosts and step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

22. Analytical method according to claim 21 characterised in that one of two cell hosts infected in step (g) expresses the CCR5 receptor and the other expresses the CXCR4 receptor.

23. Analytical method according to claim 1, 2, 11, 12 or 15 consisting of determining the susceptibility of an **HIV** virus to an inhibiting compound targeting **HIV**-1 co-receptors, characterised in that said inhibiting compound targeting **HIV**-1 co-receptors is added or not, possibly at different concentrations, during the culture step (e), in that the infection in step (f) is performed on two separate cell hosts and in that step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

24. Analytical method according to claim 1, 2, 11, 12 or 17 consisting of analysing the tropism of an **HIV** virus for a cell receptor, characterised in that the infection in step (f) with the viral particles obtained in step (c) is performed on two separate cell hosts and step (g) comprises a comparison of the expression of the marker gene by each of the two separate cell hosts.

25. Analytical method according to claim 1, 2, 11, 12 or 17 consisting of analysing the susceptibility of an **HIV** virus to an inhibiting compound targeting **HIV**-1 co-receptors, characterised in that said inhibiting compound targeting **HIV**-1 co-receptors is added, possibly at different concentrations, during the culture in step (d), in that the infection in step (f) with the viral particles in step (e) is performed on two separate cell hosts and in that step (g) comprises the comparison of the expression of the marker gene by each of the two separate cell hosts.

26. Analytical method according to any of claims 1 to 17 consisting of determining the infectivity or replicative capacity of an **HIV** virus characterised in that step (g) comprises the comparison of the expression of the marker gene by the second cell host infected with the viral particles obtained by applying steps (a) to (f) to a biological specimen from a patient, and the expression of the marker gene by the same second cell host infected with the reference viral particles obtained by applying steps (a) to (f) to a specimen containing a reference virus.

27. Analytical method according to claim 26 characterised in that the reference viral particles from a reference virus are viral particles obtained by applying steps (a) to (f) to a biological specimen from the

same patient at an earlier stage of treatment or before said treatment.

28. Analytical method according to claims 1 to 17 consisting of determining the susceptibility of an **HIV** virus to hydroxyurea, characterised in that hydroxyurea is added or not, possibly at different concentrations, either during the culture step (e), or to the second cell host, before the infection of said host in step (f) and in that step (g) comprises the comparison of the expression of the marker gene with and without hydroxyurea.

29. Analytical method according to any of claims 1 to 28 characterised in that the culture step (c) is performed during a period ranging from 12 hours to 72 hours, preferentially from 24 hours to 48 hours.

30. A kit for implementing the method according to any of claims 1 to 29 characterised in that it comprises: i) a pair of primers bordering a nucleic acid sequence of the viral genomic liable to comprise at least one mutation, ii) a vector comprising the parts of an **HIV** virus genome required for viral replication except for the segment amplified with the primers defined in (i) and the gene coding for the envelope protein, iii) a second vector comprising a gene coding for envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

31. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 1 and SEQ ID No: 2 SEQ ID No: 3 and SEQ ID No: 4 ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 protease ranging from the residues 1505 to 2565 inclusive, deleted from the envelope region and comprising a single M1uI restriction site, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the produces and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

32. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 5 and SEQ ID No: 7 SEQ ID No: 6 and SEQ ID No: 8 ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 reverse transcriptase ranging from the residues 2618 to 2872 inclusive, and comprising a single M1uI restriction site, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

33. A kit according to claim 30 characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 9 and SEQ ID No: 10 SEQ ID No: 11 and SEQ ID No: 12 ii) a retroviral vector deleted from the region of the pol reading frame coding for **HIV**-1 integrase ranging from the residues 4228 to 5093 inclusive and the region coding for the viral envelope between the positions 6343 and 7611 inclusive, iii) a pseudotype virus with a gene coding for an envelope protein, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

34. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 13 and SEQ ID No: 14 SEQ ID No: 15 and SEQ ID No: 16 ii) a retroviral vector deleted from the entire region coding for the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 7745 to 8263 inclusive, and comprising a single MluI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

35. A kit according to claim 30, characterised in that it comprises: i) the sequence primers: (SEQ ID No: 23) and (SEQ ID No: 24) (SEQ ID No: 25) and the mixture of primers (SEQ ID No: 26) and (SEQ ID No: 27), ii) a retroviral vector deleted from the entire region coding for the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 7745 to 8263 inclusive, and comprising a single MluI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

36. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 17 and SEQ ID No: 14 SEQ ID No: 18 and SEQ ID No: 16 ii) a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 6480 to 8263 inclusive, and comprising a single MulI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated by viral particles, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

37. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 17 and SEQ ID No: 24 SEQ ID No: 18 and SEQ ID No: 26 and SEQ ID No: 27 ii) a retroviral vector deleted from the entire region coding for the majority of the gp120 sub-unit and the extracellular portion of the **HIV**-1 envelope gp41 sub-unit, ranging from the residues 6480 to 8263 inclusive, and comprising a single MulI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated by viral particles, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect tho expressed marker

38. A kit according to claim 30, characterised in that it comprises: i) the sequence primer pairs: SEQ ID No: 19 and SEQ ID No: 20 SEQ ID No: 21 and SEQ ID No: 22 ii) a retroviral vector deleted from the region, coding for the domains ranging from the loop V1 to the loop V3 of the **HIV**-1 envelope, ranging from 6617 to 7250 inclusive, and comprising a single NheI restriction site, iv) a first cell host liable to be infected by an **HIV** virus, v) a second cell host liable to be infected by an **HIV** virus and comprising a marker gene that can only be activated following viral infection, vi) the products and reagents required to carry out PCR amplification, vii) the products and reagents used to detect the expressed marker.

therefor.
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National Jewish Medical and Research Center (U.S. corporation)
US 2002091073 A1 20020711
APPLICATION: US 2001-881573 A1 20010613 (9)
PRIORITY: US 1995-9460P 19951229 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a method to limit infection by an immunodeficiency virus. The method includes inhibiting an immunodeficiency virus protein which regulates apoptosis in cells. Also disclosed are methods to identify compounds that regulate cellular inhibitors of apoptosis in cells infected with an immunodeficiency virus and compounds identified thereby.

CLM What is claimed is:

1. A method to limit infection by an immunodeficiency virus, comprising inhibiting an immunodeficiency virus protein which regulates apoptosis in cells.

2. The method of claim 1, wherein said immunodeficiency virus is **HIV**.

3. The method of claim 2, wherein said **HIV** protein is selected from the group consisting of **Vpr**, Tat, Vif, Nef, Env, Gag and Vpu.

4. The method of claim 2, wherein said **HIV** protein is **Vpr**.

5. The method of claim 1, wherein said step of inhibiting comprises administering to a cell an inhibitor of an immunodeficiency virus protein which regulates apoptosis in cells.

6. The method of claim 5, wherein said cell is a T cell.

7. The method of claim 6, wherein said T cell is CD4+ or CD8+.

8. The method of claim 1, further comprising inhibiting non-immunodeficiency virus induced cellular apoptosis of uninfected bystander cells.

9. The method of claim 8, wherein said step of inhibiting non-immunodeficiency virus induced cellular apoptosis of uninfected bystander cells comprises regulating a compound selected from the group consisting of Lck, cAMP, Protein kinase A, cdc2, cysteine proteases, proteins of the TNF receptor family, p53, Ras, Raf, MEKK1, Jun kinases, bcl-2 and oxygen radicals.

10. The method of claim 1, further comprising administering an inhibitor of immunodeficiency virus replication in immunodeficiency virus infected cells.

11. The method of claim 10, wherein said immunodeficiency virus is **HIV** and said inhibitor is selected from the group consisting of nucleoside analogues, non-nucleoside analogues, protease inhibitors, chemokine receptor inhibitors, **antibodies** specific for gp160, **antibodies** specific for gp120, CD4-gp160 protein complexes and gp120-CD4 protein complexes.

12. The method of claim 1, wherein said step of inhibiting is conducted in vivo.

13. The method of claim 1, wherein said step of inhibiting is conducted ex vivo.

14. The method of claim 1, wherein said step of inhibiting comprises systemically administering an inhibitor of an immunodeficiency virus

protein which regulates apoptosis in cells.

15. A method to identify a compound that regulates a cellular inhibitor of apoptosis in cells infected with an immunodeficiency virus, said method comprising: (a) contacting a putative regulatory compound with cells infected with an immunodeficiency virus under conditions in which, in the absence of said compound, apoptosis of said cells is inhibited; and (b) assessing the ability of said putative regulatory compound to regulate apoptosis in said cells, wherein a difference in the rate of apoptosis between infected cells contacted with said compound compared to infected cells not in contact with said compound indicates that said compound regulates a cellular inhibitor of apoptosis in cells infected with said immunodeficiency virus.

16. The method of claim 15, wherein said immunodeficiency virus is **HIV**.

17. The method of claim 15, wherein said cells comprise immunodeficiency virus infected T cells.

18. The method of claim 15, wherein said T cells comprise CD4+ or CD8+ T cells.

19. The method of claim 15, wherein said step of assessing comprises determining the ability of said putative regulatory compound to induce apoptosis in said cells.

20. The method of claim 19, wherein said immunodeficiency virus is **HIV** and said step of assessing comprises determining the ability of said putative regulatory compound to inhibit an **HIV** protein selected from the group consisting of **Vpr**, Tat, Vif, Nef, Env, Gag and Vpu.

21. The method of claim 19, wherein said immunodeficiency virus is **HIV** and said step of assessing comprises determining the ability of said putative regulatory compound to inhibit the **HIV** protein **Vpr**.

22. The method of claim 15, wherein said step of assessing comprises a step selected from the group consisting of (1) determining the extent of a morphological change in a cell selected from the group consisting of progressive contraction of cell volume with the preservation of the integrity of cytoplasmic organelles and condensation of chromatin; (2) determining the extent of DNA cleavage; and (3) determining membrane permeability.

23. A compound identified by the method of claim 15.

24. A method to identify a compound that regulates a cellular inhibitor of apoptosis in cells infected with an immunodeficiency virus, said method comprising: (a) contacting a putative regulatory compound with an immunodeficiency virus apoptosis inhibitor protein; and (b) assessing the ability of said putative regulatory compound to regulate the activity of said immunodeficiency virus apoptosis inhibitor protein.

25. The method of claim 24, wherein said immunodeficiency virus is **HIV**.

26. The method of claim 25, wherein said **HIV** apoptosis inhibitor protein is selected from the group consisting of **Vpr**, Tat, Vif, Nef, Env, Gag and Vpu.

27. The method of claim 24, wherein said **HIV** apoptosis inhibitor protein is **Vpr** protein.

28. The method of claim 24, wherein said step of assessing comprises determining the ability of said putative regulatory compound to regulate an immunodeficiency virus apoptosis inhibitor protein by a mechanism selected from the group consisting of degrading said protein, inhibiting transcription of a gene encoding said protein, inhibiting translation of

said protein, inhibiting activation of said protein, and inhibiting the interaction of said protein with another protein.

L16 ANSWER 22 OF 26 USPATFULL on STN
2002:168084 Receptor-binding pocket mutants of influenza a virus hemagglutinin for use in targeted gene delivery.
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The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)
US 6416997 B1 20020709
APPLICATION: US 2000-525392 20000315 (9)
PRIORITY: US 1997-59239P 19970918 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to a lipid containing vector capable of fusing to a cell membrane and delivering a compounds contained therein to a cell, and methods of use thereof.

CLM What is claimed is:

1. A lipid-containing vector which fuses to a cell membrane, said vector comprising a mutant hemagglutinin, wherein said hemagglutinin comprises a mutation in the receptor binding pocket of said hemagglutinin, wherein said mutation substantially abrogates binding of said hemagglutinin to a sialic acid containing receptor, and further wherein said mutation does not affect the fusogenic capacity of said hemagglutinin.

2. The vector of claim 1, wherein said hemagglutinin is an influenza A virus hemagglutinin.

3. The vector of claim 2, wherein said mutant hemagglutinin comprises a mutation in at least one amino acid in the receptor-binding pocket of said influenza A virus hemagglutinin.

4. The vector of claim 2, wherein the amino acid sequence of said mutant hemagglutinin differs from the amino acid sequence of wild type influenza A virus hemagglutinin in at least one of histidine-17, aspartic acid-112, threonine-115, glutamine-190, and leucine-226.

5. The vector of claim 4, wherein the amino acid sequence of said mutant hemagglutinin differs from the amino acid sequence of wild type influenza A virus hemagglutinin in at least one of histidine-17 and aspartic acid-112, and further in at at least one of threonine-115, glutamine-190, and leucine-226.

6. The vector of claim 3, wherein said mutant hemagglutinin is selected from the group consisting of HA(T155S), HA(E190D), HA(L226V), HA(E190D, L226V), HA(T155S,L226V), HA(T155S,L226V,H17Q), HA(T155S,L226V,D112G), and HA(T155S,E190D).

7. The vector of claim 1, further comprising a targeting molecule.

8. The vector of claim 7, wherein said targeting molecule is selected from the group consisting of a viral envelope protein, an **antibody**, an **antibody** domain, an antigen, a T-cell receptor, a cell surface receptor, a cell surface adhesion molecule, a major histocompatibility locus protein, a chimeric protein comprising at least a portion of Myc protein, a chimeric protein comprising at least a portion of Tva protein, a chimeric protein comprising at least a portion of EGF, and a peptide selected by phage display that binds specifically to a defined cell.

9. The vector of claim 1, wherein said vector comprises at least one additional component.

10. The vector of claim 9, wherein said additional component is selected

from the group consisting of a nucleic acid, an antisense nucleic acid, a gene, a protein, a peptide, a **Vpr** protein, an enzyme, an intracellular antagonist of **HIV**, a radionuclide, a cytotoxic compound, an antiviral agent, and an imaging agent.

11. The vector of claim 1, wherein said vector is selected from the group consisting of an enveloped virus and a liposome.

12. A method of producing a lipid-containing vector, said method comprising pseudotyping an enveloped virus with a mutant influenza A virus hemagglutinin, wherein said mutant hemagglutinin comprises at least one amino acid substitution at residues threonine-115, glutamine-190, and leucine-226 in the receptor binding pocket of said hemagglutinin, and further wherein said substitution substantially abrogates binding of said hemagglutinin to a sialic acid containing receptor, and co-pseudotyping said virus with a targeting molecule.

13. The method of claim 12, wherein said vector comprises an additional component.

14. The method of claim 12, wherein said amino acid substitution is selected from the group consisting of a change from threonine to serine at residue 155, a change from glutamine to asparagine at residue 190, and a change from leucine to valine at residue 226.

15. The method of claim 12, wherein said vector comprises an amino acid substitution from threonine to serine at residue 155, and a second amino acid substitution from leucine to valine at residue 226.

16. The method of claim 12, wherein said targeting molecule is selected from the group consisting of a viral envelope protein, an **antibody**, an **antibody**-domain, an antigen, a T-cell receptor, a cell surface receptor, a cell surface adhesion molecule, a major histocompatibility locus protein, a chimeric protein comprising at least a portion of Myc protein, a chimeric protein comprising at least a portion of Tva protein, a chimeric protein comprising at least a portion of EGF, and a peptide selected by phage display that binds specifically to a defined cell.

17. The method of claim 16, wherein said targeting molecule is a chimeric protein comprising at least a portion of Myc, at least a portion of Tva, and at least a portion of EGF.

18. The method of claim 13, wherein said additional component is selected from the group consisting of a nucleic acid, an antisense nucleic acid, a gene, a protein, a peptide, a **Vpr** protein, an enzyme, an intracellular antagonist of **HIV**, a radionuclide, a cytotoxic compound, an antiviral agent, and an imaging agent.

19. An isolated nucleic acid encoding an influenza A virus hemagglutinin, wherein said nucleic acid comprises a mutation in the receptor binding pocket of said hemagglutinin, wherein said mutation substantially abrogates binding of said hemagglutinin to a sialic acid containing receptor, and further wherein said mutation does not affect the fusogenic capacity of said hemagglutinin.

20. The isolated nucleic acid of claim 19, wherein said mutation effects a conservative amino acid substitution.

21. The isolated nucleic acid of claim 20, wherein said conservative amino acid is selected from the group consisting of threonine-155, glutamine-190, and leucine-226.

22. An isolated influenza A virus hemagglutinin wherein said hemagglutinin comprises a mutation which substantially abrogates binding of said hemagglutinin to a sialic acid containing receptor and further

wherein said mutation does not affect the fusogenic capability of said hemagglutinin.

23. A pseudotyped murine leukemia virus (MLV) comprising a mutant influenza A hemagglutinin, wherein said mutant hemagglutinin comprises a first mutation comprising a change from threonine to serine at amino acid 155, and further wherein said mutant hemagglutinin comprises a second mutation comprising a change from leucine to valine at amino acid 226, wherein said pseudotyped MLV expresses said mutant hemagglutinin and wherein said mutant hemagglutinin is in the envelope of said pseudotyped MLV.

24. The pseudotyped MLV of claim 23, wherein said hemagglutinin further comprises a third mutation comprising an amino acid substitution which causes said mutant hemagglutinin to undergo low-pH induced conformational changes to a fusogenic form.

25. The pseudotyped MLV of claim 24, wherein said third mutation comprises an amino acid substitution selected from the group consisting of a substitution from histidine to glutamine at amino acid 17 and a substitution from asparagine to glycine at amino acid 112.

26. A composition comprising a co-pseudotyped enveloped virus expressing a mutant hemagglutinin and a targeting molecule wherein said co-pseudotyped virus binds to a target cell expressing a receptor for said targeting molecule and further wherein said mutant hemagglutinin causes said virus to fuse with said target cell.

27. An isolated mammalian cell comprising the pseudotyped virus of claim 23.

28. An isolated mammalian cell comprising the co-pseudotyped virus of claim 26.

29. A method of targeting delivery of a component to a desired cell, said method comprising inserting a mutant hemagglutinin and a targeting molecule on the surface of a vector, wherein said targeting molecule mediates binding of said vector to a targeting molecule-specific receptor on said cell, and further wherein said mutant hemagglutinin mediates membrane fusion of said vector with the membrane of said cell, thereby delivering said component to said cell.

30. The method of claim 29, wherein said component is selected from the group consisting of a nucleic acid, an antisense nucleic acid, a gene, a protein, a peptide, a **Vpr** protein, an enzyme, an intracellular antagonist of **HIV**, a radionuclide, a cytotoxic compound, an antiviral agent, and an imaging agent.

L16 ANSWER 23 OF 26 USPATFULL on STN
2002:119333 Non-infectious, protease defective **HIV** particles and nucleic acid molecules encoding therefor.
Luftig, Ronald B., Metairie, LA, UNITED STATES
The Board of Supervisors of Louisiana State University (U.S. corporation)
US 2002061313 A1 20020523
APPLICATION: US 2001-919124 A1 20010730 (9)
PRIORITY: US 1997-43047P 19970404 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed toward mutated DNA, proteins, or protein fragments and particles from the L-2 cell line. The invention is also directed to diagnostic, prophylactic and therapeutic methods of making and using the DNA, proteins and particles.

CLM What is claimed is:
1. A substantially pure nucleic acid molecule or fragment thereof that encodes a truncated Nef protein comprising a nucleic acid sequence that

encodes substantially the same sequence of amino acid residues 1 through 56 of an **HIV**-1 Nef protein.

2. The nucleic acid molecule of claim 1 wherein said nucleic acid sequence is selected from the group consisting of: a nucleic acid molecule that encodes substantially the same amino acid sequence set forth in SEQ ID NO:46; and a nucleic acid molecule that encodes substantially the same sequence of amino acid residues 1 through 56 of an **HIV**-1 Nef peptide wherein amino acid residue 54 is aspartic acid.

3. The nucleic acid molecule of claim 2 wherein said nucleic acid sequence is selected from the group consisting of: (a) nucleotides 1 through 168 of SEQ ID NO:45; and (b) nucleotides 1 through 168 of an **HIV**-1 Nef peptide wherein nucleotide 161 is adenine.

4. A substantially pure fragment of the nucleic acid molecule of claim 3 comprising a nucleic acid sequence that has one or more of the following amino acid residues of SEQ ID NO:45: guanine at nucleotide 43, adenine at nucleotide 56, thymine at nucleotide 98, adenine at nucleotide 111, and adenine at nucleotide 161 and can be used to specifically detect the nucleic acid molecule of claim 3(a).

5. A substantially pure mutated **HIV**-1 Nef protein fragment, comprising a peptide encoded by the nucleic acid molecule fragment of claim 4.

6. An **antibody** or fragment thereof that specifically binds to the protein fragment of claim 5.

7. A substantially pure truncated Nef protein or fragment thereof comprising substantially the same sequence of amino acid residues 1 through 56 of an **HIV**-1 Nef protein.

8. The truncated Nef protein of claim 7 wherein said sequence is selected from the group consisting of: substantially the same amino acid sequence set forth in SEQ ID NO:46; and substantially the same sequence of amino acid residues 1 through 56 of an **HIV**-1 Nef protein wherein amino acid residue 54 is aspartic acid.

9. The truncated protein of claim 8 wherein said amino acid sequence is selected from the group consisting of: amino acid residues 1 through 56 of SEQ ID NO:46; and amino acid residues 1 through 56 of an **HIV**-1 Nef peptide wherein amino acid residue 54 is aspartic acid.

10. An **antibody** or fragment thereof that specifically binds to the mutated protein of claim 7, 8 or 9.

11. A substantially pure nucleic acid molecule or fragment thereof that encodes a mutated **HIV**-1 Env gp41 protein containing an arginine at amino acid residue 660.

12. The nucleic acid molecule of claim 11 wherein nucleotide 1979 is guanine.

13. A substantially pure fragment of the nucleic acid molecule of claim 12 comprising a portion of said nucleic acid molecule that contains said guanine at nucleotide 1979 and can be used to specifically detect the nucleic acid molecule of claim 12.

14. A substantially pure mutated **HIV**-1 Env gp41 protein fragment, comprising a peptide encoded by the nucleic acid molecule fragment of claim 13.

15. An **antibody** or fragment thereof that specifically binds to the protein fragment of claim 14.

16. A substantially pure mutated **HIV**-1 Env gp41 protein or fragment

cluster comprising an HIV-1 Env gp41 protein containing an arginine at amino acid residue 660.

17. An **antibody** or fragment thereof that specifically binds to the protein of claim 16.

18. A substantially pure nucleic acid molecule or fragment thereof that encodes a mutated **HIV**-1 Env gp120 protein selected from the group consisting of: (a) a nucleic acid sequence that encodes an **HIV**-1 Env gp120 protein wherein: amino acid residue 143 is either serine or arginine; up to eight amino acids at amino acid residues 144 through 151 in the V1 domain are deleted; and amino acid residue 153 is either methionine or isoleucine; and (b) a nucleic acid sequence that encodes an **HIV**-1 Env gp120 protein wherein: amino acid residue 187 is either isoleucine or valine; and one or two amino acids at amino acid residues 192 and 193 in the V2 domain are deleted.

19. A substantially pure fragment of the nucleic acid molecule of claim 18 comprising a portion of the nucleic acid molecule that contains said deletions and that can be used to specifically detect the nucleic acid molecule of claim 18.

20. A substantially pure mutated **HIV**-1 Env gp120 protein fragment, comprising a peptide encoded by the nucleic acid molecule fragment of claim 19.

21. An **antibody** or fragment thereof that specifically binds to the protein fragment of claim 20.

22. A substantially pure mutated **HIV**-1 Env gp120 protein or fragment thereof selected from the group consisting of: (a) the amino acid sequence of an **HIV**-1 Env gp120 protein wherein amino acid residue 143 is either serine or arginine; up to eight amino acids at amino acid residues 144 through 151 in the V1 domain are deleted; and amino acid residue 153 is either methionine or isoleucine; and (b) the amino acid sequence of an **HIV**-1 Env gp120 protein wherein amino acid residue 187 is either isoleucine or valine; and one or two amino acids at amino acid residues 192 and 193 in the V2 domain are deleted.

23. An **antibody** or fragment thereof that specifically binds to the protein of claim 22.

24. A substantially pure nucleic acid molecule or fragment thereof that encodes a truncated **HIV**-1 **Vpr** protein comprising a nucleic acid sequence encoding substantially the same amino acid residues 1 through 17 of an **HIV**-1 **Vpr** protein.

25. The nucleic acid molecule of claim 24 wherein said nucleic acid sequence comprises nucleotides 1 through 51 of an **HIV**-1 **vpr** gene.

26. A substantially pure truncated **HIV**-1 **Vpr** protein or fragment thereof comprising substantially the same amino acid residues 1 through 17 of an **HIV**-1 **Vpr** protein.

27. An **antibody** or fragment thereof that specifically binds to the protein of claim 26.

28. A substantially pure nucleic acid molecule or fragment thereof that encodes a mutated and truncated **HIV**-1 Pol protease protein comprising a nucleic acid sequence encoding substantially the same sequence of amino acid residues 1 through 13 of a wild-type **HIV**-1 Pol protease protein and amino acid residues 14 though 29 of SEQ ID NO:6.

29. The nucleic acid molecule of claim 26 wherein said nucleic acid sequence comprises nucleotides 1 through 41 of a wild-type **HIV**-1 Pol protease gene and nucleotides 42 though 87 of SEQ ID NO:5.

30. A substantially pure fragment of the nucleic acid molecule of claim 29, comprising a fragment of SEQ ID NO:5 at least 8 nucleotides in length and that can be used to specifically detect the nucleic acid molecule of claim 29.

31. A substantially pure mutated and truncated **HIV**-1 Pol protease protein or fragment thereof comprising substantially the same sequence of amino acid residues 1 through 13 of a wild-type **HIV**-1 Pol protease protein and amino acid residues 14 though 29 of SEQ ID NO:6.

32. An immunogen comprising an inactivated protease-defective viral **HIV**-1 particle containing one or more of the following substantially pure proteins: an **HIV**-1 Env gp120 protein comprising substantially the same protein of claim 22; a mutated **HIV**-1 Pol protease molecule comprising substantially the same sequence of amino acid residues 1 through 13 of a wild-type **HIV**-1 Pol protease protein and amino acid residues 14 though 29 of SEQ ID NO:6; a truncated **HIV**-1 Nef protein or fragment thereof comprising substantially the same sequence of amino acid residues 1 though 56 of an **HIV**-1 Nef protein; a mutated **HIV**-1 Nef protein comprising substantially the same amino acid sequence set forth in SEQ ID NO:46 or a fragment thereof comprising one or more of the following amino acid residues of SEQ ID NO:46: alanine at amino acid residue 15, lysine at amino acid residue 19, valine at amino acid residue 33, and aspartic acid at amino acid residue 54; a truncated **HIV**-1 Nef protein comprising substantially the same sequence of amino acid residues 1 through 56 of a Nef peptide wherein amino acid residue 54 is aspartic acid; a truncated **HIV**-1 **Vpr** protein or fragment thereof comprising substantially the same amino acid residues 1 through 17 of an **HIV**-1 **Vpr** protein; or an **HIV**-1 Env gp41 protein or fragment thereof comprising substantially the same amino acid sequence of an **HIV**-1 Env gp41 protein containing an arginine at amino acid residue 660.

33. The immunogen of claim 32 further comprising one or more suitable adjuvants.

34. A method of detecting a mutated **HIV**-1 gene selected from the group consisting of a nef gene, an env gp41 gene and an env gp120 gene or fragments thereof in a sample of cells, lysed cells or extracellular fluid from an individual, comprising the steps of: obtaining from an individual a sample of cells, lysed cells or extracellular fluid suspected of containing said mutated gene or fragments thereof; contacting said sample with a measured amount of the nucleic acid molecule of claims 4, 13 or 19, respectively, to hybridize with said gene or fragment thereof; and determining the presence of said hybridized molecule to detect the presence of the mutated gene or fragments thereof in said sample.

35. A method of detecting the presence of a mutated **HIV**-1 protein selected from the group consisting of a Nef protein, an Env gp41 protein and an Env gp120 protein or fragments thereof in an individual, comprising the steps of: obtaining from an individual a sample of cells, lysed cells or extracellular fluid suspected of containing a mutated **HIV**-1 protein or fragments thereof; contacting said sample with a measured amount of the **antibody** of claims 6 or 10, 15 or 17, or 21 or 23, respectively, to form a complex of the **antibody** and said protein or fragment thereof; and determining the presence of said complex to detect the presence of the mutated protein or fragments thereof in said sample.

36. A method of reducing the severity of **HIV**-1 infections in individuals, comprising contacting protease-defective viral particles containing one or more mutated **HIV**-1 Nef proteins, mutated **HIV**-1 Env gp41 proteins, or mutated **HIV**-1 Env gp120 proteins or fragments thereof present on cells, in lysed cells or in extracellular fluid with

an effective amount of the **antibody** of claims 9 or 10, 15 or 17, or 21 or 23, respectively, to inhibit the activity of said proteins or fragments thereof.

37. The method of claim 36 wherein said **antibody** is attached to a moiety selected from the group consisting of radioactive moieties, chemotherapeutic moieties and chemotoxic moieties.

38. A method of reducing or preventing apoptotic cell lysis in an **HIV**-1 seropositive or seronegative individual, comprising administering an immunologically effective amount of the immunogen of claim 32 or 33 to the individual.

L16 ANSWER 24 OF 26 USPATFULL on STN
2002:112895 RIBOZYMES TARGETING THE RETROVIRAL PACKAGING SEQUENCE EXPRESSION CONSTRUCTS AND RECOMBINANT RETROVIRUSES CONTAINING SUCH CONSTRUCTS.
SYMONDS, GEOFFREY P., ROSE BAY, AUSTRALIA
SUN, LUN-QUAN, RYDE, AUSTRALIA
US 2002058636 A1 20020516
APPLICATION: US 1995-375291 A1 19950118 (8)
PRIORITY: WO 1995-IB50 19950105
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed to a synthetic non-naturally occurring oligonucleotide compound which comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus. Preferably, the viral packaging sequence is a retrovirus packaging sequence or the **HIV**-1 Psi packaging sequence. The RNA virus may be **HIV**-1, Feline Leukemia Virus, Feline Immunodeficiency Virus or one of the viruses listed in Table I. The conserved catalytic region may be derived from a hammerhead ribozyme, a hairpin ribozyme, a hepatitis delta ribozyme, an RNAase P ribozyme, a group I intron, a group II intron. The invention is also directed to multiple ribozymes, combinations of ribozymes, with or without antisense, and combinations of ribozymes, with antisense, and TAR decoys, polyTARs and RRE decoys targeted against the RNA virus. Vectors are also described. Further, methods of treatment and methods of use both in vivo and ex vivo are described.

CLM What is claimed is:

1. A synthetic non-naturally occurring oligonucleotide compound which comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus.

2. The compound of claim 1, wherein the viral packaging sequence of is a retrovirus packaging sequence.

3. The compound of claim 1, wherein the packaging sequence is the **HIV**-1 Psi packaging sequence.

4. The compound of claim 1, wherein the RNA virus is a Feline Leukemia Virus.

5. The compound of claim 1, wherein the RNA virus is a Feline Immunodeficiency Virus.

6. The compound of claim 1 having the structure: ##STR4## wherein each X represents a nucleotide which is the same or different and may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'--AAG . . . AGUCX--5' defines the conserved catalytic region; wherein each of $(X)_{nA}$ and $(X)_n$, defines the nucleotides whose sequence is

capable of hybridizing with the predetermined target sequence within the packaging sequence of the RNA virus; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; wherein a represents an integer which defines a number of nucleotides with the proviso that a may be 0 or 1 and if 0, the A located 5' of $(X)_a$ is bonded to the X located 3' of $(X)_a$; wherein each of m and m' represents an integer which is greater than or equal to 1; wherein $(X)_b$ represents an oligonucleotide and b represents an integer which is greater than or equal to 2.

7. The compound of claim 1 having the structure: ##STR5## wherein each X is the same or different and represents a ribonucleotide or a deoxyribonucleotide which may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'--AAG . . . AGUCX--5 defines the conserved catalytic region; wherein each of $(X)_{nA}$ and $(X)_{n'}$ defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of an RNA virus; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein m represents an integer from 2 to 20; and wherein none of the nucleotides $(X)_m$ are Watson-Crick base paired to any other nucleotide within the compound.

8. The compound of claim 1 having the structure: ##STR6## wherein each X is the same or different and represents a ribonucleotide or a deoxyribonucleotide which may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'$(X)_{P4}$ . . . $(X)_{P1--5}$' defines the conserved catalytic region; wherein each of $(X)_{F4}$ and $(X)_{F3}$ defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of an RNA virus; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein F3 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F3 is greater than or equal to 3; wherein F4 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F4 is from 3 to 5; wherein each of $(X)_{P1}$ and $(X)_{P4}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P4}$ base-pairs with 3-6 bases of $(X)P_1$; wherein P1 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that P1 is from 3 to 6 and the sum of P1 and F4 equals 9; wherein each of $(X)_{P2}$ and $(X)_{P3}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P2}$ base-pairs with at least 3 bases of $(X)_{P3}$; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; and wherein $(X)_{L2}$ represents an oligonucleotide which may be present or absent with the proviso that L2 represents an integer which is greater than or equal to 3 if $(X)_{L2}$ is present.

9. The compound of claim 1, wherein the nucleotides whose sequences define a conserved catalytic region are from the hepatitis delta virus conserved region.

10. The compound of claim 1, wherein the nucleotides whose sequences define a conserved catalytic region contain the sequence NCCA at its 3' terminus.

11. A synthetic non-naturally occurring oligonucleotide compound which comprises two or more domains which may be the same or different wherein each domain comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus.

12. The compound of claim 1 and further comprising a covalently linked antisense nucleic acid compound capable of hybridizing with a predetermined sequence, which may be the same or different, within a packaging sequence of the RNA virus.

13. The compound of claim 1, wherein the nucleotides are capable of hybridizing with the 243, 274, 366 or 553 target sequence in the MOMLV, and site 749 in the **HIV** Psi packaging site.

14. A compound comprising the compound of claim 1, and further comprising at least one additional synthetic non-naturally occuring oligonucleotide compound with or without an antisense molecule covalently linked, and targeted to a different gene of the RNA virus genome.

15. The compound of claim 14, wherein the RNA virus is **HIV** and the different region of the **HIV** genome is selected from the group consisting of long terminal repeat, 5' untranslated region, splice donor-acceptor sites, primer binding sites, 3' untranslated region, gag, pol, protease, integrase, env, tat, rev, nef, vif, **vpr**, vpu, vpx, or tev region.

16. The compound of claim 15, wherein the nucleotides are capable of hybridizing with the 243, 274, 366 or 553 target sites or combination thereof in the MOMLV and site 749 in the **HIV** Psi packaging site and the nucleotides of the additional compound are capable of hybridizing with the 5792, 5849, 5886, or 6042 target sites or combination thereof in the **HIV** tat region.

17. A composition which comprises the compound of claims 1 or 14 in association with a pharmaceutically, veterinarially, or agriculturally acceptable carrier or excipient.

18. A composition which comprises the compound of claim 1, with or without antisense, and further comprises a TAR decoy, polyTAR or a RRE decoy.

19. A method for producing the compound of claim 1 which comprises the steps of: (a) ligating into a transfer vector comprised of DNA, RNA or a combination thereof a nucleotide sequence corresponding to the compound; (b) transcribing the nucleotide sequence of step (a) with an RNA polymerase; and (c) recovering the compound.

20. A transfer vector comprised of RNA or DNA or a combination thereof containing a nucleotide sequence which on transcription gives rise to the compound of claim 1.

21. The transfer vector of claim 20, wherein the transfer vector comprises the **HIV** long terminal repeat, an adenovirus associated transfer vector, an SV40 promoter, Mo-MLV, or an amphotropic retrovirus vector.

22. The transfer vector of claim 20 further comprising a sequence directing the oligonucleotide compound to a particular organ or cell in vivo or a particular region within the cell.

23. A composition which comprises the transfer vector of claim 20 in association with a pharmaceutically, veterinarially or agriculturally acceptable carrier or excipient.

24. A prokaryotic or eukaryotic cell comprising a nucleotide sequence which is, or on transcription give s rise to the compound of claim 1.

25. The cell of claim 24, wherein the cell is a eukaryotic cell.

26. The eukaryotic cell of claim 25, wherein the cell is an animal cell.

27. The eukaryotic cell of claim 25, wherein the cell is a hematopoietic stem cell which gives rise to progenitor cells, more mature, and fully mature cells of all the hematopoietic cell lineages.

28. The eukaryotic cell of claim 25, wherein the cell is a progenitor cell which gives rise mature cells of all the hematopoietic cell lineages.

29. The eukaryotic cell of claim 25, wherein the cell is a committed progenitor cell which gives rise to a specific hematopoietic lineage.

30. The eukaryotic cell of claim 25, wherein the cell is a T lymphocyte progenitor cell.

31. The eukaryotic cell of claim 25, wherein the cell is an immature T lymphocyte.

32. The eukaryotic cell of claim 25, wherein the cell is a mature T lymphocyte.

33. The eukaryotic cell of claim 25, wherein the cell is a myeloid progenitor cell.

34. The eukaryotic cell of claim 25, wherein the cell is a monocyte/macrophage cell.

35. The use of the compound of claims 1 to protect hematopoietic stem cells, progenitor cells, committed progenitor cells, T lymphocyte progenitor cells, immature T lymphocytes, mature T lymphocytes, myeloid progenitor cells, or monocyte/macrophage cells.

36. A method to suppress **HIV** in an AIDS patient which comprises the introduction of the transfer vector of claim 20 into hematopoietic cells thereby rendering the cells resistant to **HIV** so as to thereby suppress **HIV** in an AIDS patient.

37. The method of claim 36, wherein the introduction is ex vivo and the cells are autologous or heterologous cells.

38. The method of claim 36, wherein the introduction is ex vivo and the cells are transplanted without myeloablation.

39. The method of claim 36, wherein the introduction is ex vivo and the cells are transplanted with myeloablation.

40. The method of claim 37, wherein the cells are also treated with an additional agent to inhibit or eliminate **HIV**-1 replication.

41. The method of claim 40, wherein the additional agent is a neutralizing **antibody** such as IgGlbl2; a nucleoside analogues such as

inhibitors such as nevirapine, delavirdine, lamivudine (3-TC), loviride; or a protease inhibitors such as saquinavir.

42. A method for protecting an individual from **HIV** infection which comprises incorporation of the transfer vector of claim 20 into the individual's cells thereby protecting that individual from the effects of high levels of the virus.

L16 ANSWER 25 OF 26 USPATFULL on STN
1999:136701 Altered major histocompatibility complex (MHC) determinant and method of using the determinant.
Mottez, Estelle, Paris, France
Abastado, Jean-Pierre, Paris, France
Kourilsky, Philippe, Paris, France
Institut Pasteur and Institut Nationale de la Sante et de la Recherche Medicale, Paris, France (non-U.S. corporation)
US 5976551 19991102
APPLICATION: US 1995-484905 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An altered MHC class II determinant comprises $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, domains of a mammalian MHC class II locus in which the domains are covalently linked to form a construct comprising the $\beta_2$ -$\alpha_2$ -$\alpha_1$ $\beta_1$ domains in sequence. The altered class II determinants can be associated with an antigen to elicit an immune response. In addition, the altered determinants may be used to prepare **antibodies**. The **antibodies** so produced have various diagnostic and therapeutic uses.

CLM What is claimed is:
1. A composition, which is capable of eliciting anti-MHC **antibodies**, comprising an antigen associated with an altered MHC class II determinant comprising $\alpha_1$, $\alpha_2$, $\beta_1$, and $\beta_2$ polypeptide domains encoded by a mammalian MHC class II locus wherein the domains of the altered MHC Class II determinant are covalently linked to form a polypeptide comprising the $\beta_2$, $\alpha_2$, $\alpha_1$, and $\beta_1$ domains in sequence, and wherein the antigen-MHC complex so formed is recognizable by a T cell receptor.

2. Composition as claimed in claim 1, wherein said antigen is a peptide containing from five to twenty amino acid residues.

3. Composition as claimed in claim 2, wherein said peptide is a peptide of **HIV**-1 wherein said peptide of **HIV**-1 is selected from the group consisting of a gag, an env, a nef, a vif, a ref, and a **vpr** peptide.

4. Composition as claimed in claim 3, wherein said peptide is env, gag, vif, or **vpr** of **HIV**.

5. Composition as claimed in claim 2, wherein said peptide is an octamer.

6. A composition as claimed in claim 2, wherein said peptide is an nanomer.

7. A composition as claimed in claim 2, wherein said peptide is an decamer.

8. Composition as claimed in claim 3, wherein said peptide of **HIV**-1 is selected from the group of peptides consisting of SEQ. ID. No.19, SEQ. ID. No.20, SEQ. ID. No.21, SEQ. ID. No.22, SEQ. ID. No.23, SEQ. ID. No.24, SEQ. ID. No.25, SEQ. ID. No.26, SEQ. ID. No.27, SEQ. ID. No.28,

SEQ. ID. No.33, SEQ. ID. No.34, SEQ. ID. No.35, SEQ. ID. No.36, SEQ. ID. No.37, SEQ. ID. No.38, SEQ. ID. No.39, SEQ. ID. No.40, SEQ. ID. No.41, SEQ. ID. No.42, SEQ. ID. No.43, SEQ. ID. No.44, SEQ. ID. No.45, SEQ. ID. No.46, SEQ. ID. No.47, SEQ. ID. No.48, SEQ. ID. No.49, SEQ. ID. No.50, SEQ. ID. No.51, SEQ. ID. No.52, SEQ. ID. No.53, SEQ. ID. No.54, SEQ. ID. No.55, SEQ. ID. No.56, SEQ. ID. No.57, SEQ. ID. No.58, SEQ. ID. No.128, SEQ. ID. No.129, SEQ. ID. No.130, SEQ. ID. No.131, SEQ. ID. No.132, SEQ. ID. No.133, SEQ. ID. No.134, SEQ. ID. No.135, SEQ. ID. No.136, SEQ. ID. No.137, SEQ. ID. No.138, SEQ. ID. No.139, SEQ. ID. No.140, SEQ. ID. No.141, SEQ. ID. No.142, SEQ. ID. No.143, SEQ. ID. No.144, SEQ. ID. No.145, SEQ. ID. No.146, SEQ. ID. No.147, and SEQ. ID. No.148.

L16 ANSWER 26 OF 26 USPATFULL on STN

1999:124707 Method of intracellular binding of target molecules.

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US 5965371 19991012
APPLICATION: US 1995-438190 19950509 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a method by which one can target an undesired target molecule or target antigen, preferably a protein. The method comprises the intracellular expression of an **antibody** capable of binding to the target. A DNA sequence is delivered to a cell, the DNA sequence contains a sufficient number of nucleotides coding for the portion of an **antibody** capable of binding to the target operably linked to a promoter that will permit expression of the **antibody** in the cell(s) of interest. The **antibody** is then expressed intracellularly and binds to the target, thereby disrupting the target from its normal actions.

CLM What is claimed is:

1. A method for the intracellular binding of a target antigen which comprises: (a) intracellular delivery of a nucleotide sequence containing a promoter operably linked to an **antibody** gene capable of binding to the target antigen; (b) intracellular expression of the **antibody**, wherein said **antibody** is intracellularly expressed as a functional **antibody** where said function is determined by the ability to bind to the target antigen, and wherein said **antibody** is selected from the group of **antibodies** consisting of single chain **antibodies**, single domain heavy chain and Fab; and (c) intracellular binding of the target antigen by said **antibody**.

2. The method of claim 1, wherein the **antibody** capable of binding to the target antigen is a single chain variable fragment.

3. The method of claim 1, wherein the **antibody** capable of binding to the target antigen is a single domain heavy chain.

4. The method of claim 1, wherein the **antibody** capable of binding to the target antigen is a Fab.

5. The method of claim 1, wherein the target antigen is selected from the group of antigens consisting of intermediate metabolites, sugars, lipids, autacoids, hormones, complex carbohydrates, phospholipids, nucleic acids and proteins.

6. The method of claim 1, wherein the target antigen is a hapten, an RNA sequence, a DNA sequence or a protein.

7. The method of claim 6, wherein the target antigen is a protein.

8. The method of claim 1, wherein the target antigen is a protein whose expression results in malignant cellular transformation.

9. The method of claim 8, wherein the target antigen results in malignant transformation as a result of overexpression of the protein.

10. The method of claim 8, wherein the target antigen is an HTLV-1 protein.

11. The method of claim 6, wherein the target antigen is a hapten.

12. The method of claim 1, wherein the target antigen is a viral encoded protein.

13. The method of claim 12, wherein the viral encoded protein is an **HIV** viral encoded protein.

14. The method of claim 12, wherein the **antibody** is an **antibody** capable of binding to the envelope glycoprotein or the capsid protein.

15. The method of claim 13, wherein the **antibody** is capable of binding to the envelope glycoprotein.

16. The method of claim 15, wherein the target antigen is the envelope gp160.

17. The method of claim 1, wherein the target antigen is an **HIV** provirus.

18. The method of claim 15, wherein the target protein is the envelope gp41.

19. The method of claim 6 wherein the target antigen is a TAR element or a RRE sequence.

20. The method of claim 1, wherein one uses **antibodies** to more than one target antigen.

21. The method of claim 20, wherein the target antigens are virally encoded protein and the **antibodies** are to at least two different virally encoded proteins.

22. The method of claim 21, wherein the virally encoded proteins are **HIV** encoded proteins and the **antibodies** are to at least one structural protein and at least one regulatory protein.

23. The method of claim 22, wherein the structural protein is an envelope glycoprotein and the regulatory protein is either the tat or rev protein.

24. The method of claim 23, wherein the envelope glycoprotein is gp160.

25. The method of claim 24, which further comprises an **antibody** to **HIV** gp41.

26. The method of claim 12, wherein the **antibody** is to that portion of the capsid protein involved in myristylation.

27. The method of claim 13, wherein the **antibody** is to the tat protein.

28. The method of claim 1, wherein the **antibody** gene further encodes an intracellular localization sequence.

29. The method of claim 28, wherein more than one **antibody** to the same target are used, wherein the **antibodies** have different intracellular localization sequences and target the antigen at different intracellular locations.

30. The method of claim 29, wherein the target antigen is a virally encoded antigen.

31. The method of claim 30, wherein virally encoded antigen is an **HIV** encoded antigen.

32. The method of claim 31, wherein the **HIV** encoded antigen is an envelope glycoprotein.

33. The method of claim 12, wherein the **antibody** gene further encodes an intracellular localization sequence.

34. The method of claim 33, wherein the localization sequence for the structural proteins is cytoplasmic.

35. The method of claim 33, wherein the **viral protein** is selected from the group of viral proteins comprising **HIV** tat, **HIV** rev, HTLV-1 tax, HTLV-1 rex, HTLV-2 tax, and HTLV-2 rex, and the localization sequence is a nuclear localization sequence.

36. The method of claim 13, wherein the **antibody** is to that portion of the capsid protein involved in myristylation.

37. The method of claim 12, wherein the virally encoded protein is a DNA virus encoded protein.

38. The method of claim 12, wherein the virally encoded protein is a RNA virus encoded protein.

39. The method of claim 1, wherein the target antigen is an oncogene.

40. The method of claim 1, wherein the target antigen is selected from the group consisting of sis, int-2, erbB, neu, fins, ros, kit, abl, src, ras, and erbA.

41. The method of claim 1, wherein the cell is an animal or bird cell.

42. The method of claim 41, wherein the cell is an animal cell.

43. The method of claim 42, wherein the animal is a mammalian cell.

44. A method for the intracellular binding of a target antigen, comprising: (a) introducing an **antibody** cassette into a cell, wherein said **antibody** cassette contains a nucleic acid segment encoding a light chain of an **antibody** and a nucleic acid segment encoding a heavy chain of an **antibody** operably linked to at least one promoter wherein the **antibody** cassette encodes a single chain **antibody** or Fab'; (b) intracellular expression of said **antibody** encoded by said nucleic acid segments encoding said light chain and said heavy chain; and (c) intracellular binding of said target antigen by said **antibody**.

45. The method of claim 44, wherein said nucleic acid segment encoding said light chain is linked to said nucleic acid segment encoding said heavy chain by a nucleic acid segment encoding a linker which is in-frame with nucleic acid segments to produce a single chain **antibody**.

46. The method of claim 44, wherein said linker is SEQ ID NO:1.

47. The method of claim 44, wherein the **antibody** expressed by the **antibody** cassette is a Fab'.

48. The method of claim 44, wherein the target antigen is a protein.

49. The method of claim 44, wherein the target antigen is a protein whose expression results in malignant cellular transformation.

50. The method of claim 49, wherein the target antigen results in malignant transformation as a result of overexpression of the protein.

51. The method of claim 44, wherein the target antigen is a viral-encoded protein.

52. The method of claim 51, wherein the viral encoded protein is an **HIV** viral-encoded protein.

53. The method of claim 52, wherein the target antigen is an **HIV** regulatory protein.

54. The method of claim 53, wherein the regulatory protein is the rev protein.

55. The method of claim 51, wherein the **antibody** is an **antibody** capable of binding to the envelope glycoprotein.

56. The method of claim 55, wherein the target antigen is the **HIV** envelope gp160.

57. The method of claim 44, wherein the target antigen is a TAR element or a RRE sequence.

58. The method of claim 44, wherein the cell is an animal or bird cell.

59. The method of claim 58, wherein the cell is an animal cell.

60. The method of claim 59, wherein the animal is a mammalian cell.

61. A method for the intracellular binding of a target antigen, which comprises: (a) delivery of a nucleic acid segment encoding a single chain **antibody** and a promoter operably linked to said nucleic acid segment to the interior of a cell; (b) intracellular expression of said single chain **antibody**; and (c) intracellular binding of said target antigen by said single chain **antibody**.

62. The method of claim 61, wherein the target antigen is a protein.

63. The method of claim 61, wherein the target antigen is a protein whose expression results in malignant cellular transformation.

64. The method of claim 63, wherein the target antigen results in malignant transformation as a result of overexpression of the protein.

65. The method of claim 61, wherein the target antigen is a viral-encoded protein.

66. The method of claim 65, wherein the viral encoded protein is an **HIV** viral-encoded protein.

67. The method of claim 66, wherein the target antigen is an **HIV** regulatory protein.

68. The method of claim 67, wherein the regulatory protein is the rev protein.

69. The method of claim 65, wherein the **antibody** is an **antibody** capable of binding to the envelope glycoprotein.

70. The method of claim 61, wherein the target antigen is a TAR element or a RRE sequence.

71. The method of claim 61, wherein the cell is an animal or bird cell.

72. The method of claim 71, wherein the cell is an animal cell.

73. The method of claim 72, wherein the cell is an animal or bird cell.

74. The method of claim 73, wherein the cell is an animal cell.

75. The method of claim 74, wherein the animal cell is a mammalian cell.

76. The method of claim 72, wherein the animal is a mammalian cell.

77. The method of claim 61, wherein said single chain **antibody** contains a linker between said single chain **antibody**'s variable light chain and variable heavy chain.

78. The method of claim 77, wherein said linker is SEQ ID NO:1.

79. A method for the intracellular binding of a target antigen, which comprises: (a) delivery of a nucleic acid segment containing a promoter operably linked to an **antibody** gene capable of binding to said target antigen, wherein the **antibody** expressed by said **antibody** gene does not have a secretory sequence, and wherein the **antibody** is a single chain **antibody** or a Fab'; (b) intracellular expression of said **antibody** in a form capable of binding to said target antigen; and (c) intracellular binding of said target antigen by said **antibody**.

80. The method of claim 79, wherein the **antibody** expressed by the nucleic acid segment is a Fab'.

81. The method of claim 79, wherein the target antigen is a protein.

82. The method of claim 79, wherein the target antigen is a protein whose expression results in malignant cellular transformation.

83. The method of claim 82, wherein the target antigen results in malignant transformation as a result of overexpression of the protein.

84. The method of claim 79, wherein the target antigen is a viral-encoded protein.

85. The method of claim 84, wherein the viral encoded protein is an **HIV** viral-encoded protein.

86. The method of claim 85, wherein the target antigen is an **HIV** regulatory protein.

87. The method of claim 86, wherein the regulatory protein is the rev protein.

88. The method of claim 84, wherein the **antibody** is an **antibody** capable of binding to the envelope glycoprotein.

89. The method of claim 84, wherein the target antigen is the **HIV** envelope gp160.

90. The method of claim 79, wherein the target antigen is a TAR element or a RRE sequence.

91. The method of claim 79, wherein said single chain **antibody** contains a linker between said single chain **antibody**'s variable light chain and variable heavy chain.

92. The method of claim 91, wherein said linker is SEQ ID NO:1.

93. A method for binding a target protein by an **antibody** inside a human cell at a specified location which comprises: (a) delivery to said cell of a nucleic acid segment encoding at least a variable light chain and a variable heavy chain of an **antibody** which will bind to said

protein, wherein said nucleic acid segment also encodes a localization sequence, (b) intracellular expression of said **antibody** in a form capable of binding to said target protein, wherein said **antibody** is a single chain or Fab', (c) intracellular delivery of said **antibody** to a site directed by said localization sequence, and (d) intracellular binding of said target protein at said site.

94. The method of claim 93, wherein the localization sequence is selected from the group consisting of routing signals, sorting signals, retention signals, salvage signals, and membrane topology-stop transfer signals.

95. A method for the intracellular binding of a target antigen, comprising: (a) introducing a nucleic acid segment encoding at least the heavy chain variable sequence of an **antibody** operably linked to a promoter into an animal cell, wherein the **antibody** is selected from the group consisting of single domain heavy chain, single chain, and Fab'; (b) intracellular expression of said heavy chain variable sequence; and (c) intracellular binding of said target antigen by said heavy chain variable sequence.

96. The method of claim 95, wherein the nucleic acid segment encodes only the heavy chain of a Fab.

97. The method of claim 95, wherein the nucleic acid segment encodes only the heavy chain variable sequence.

98. A method for the intracellular binding of a target antigen, comprising: (a) delivery of a nucleic acid segment containing a promoter operably linked to an **antibody** gene encoding an **antibody** capable of binding to said target antigen wherein the **antibody** contains a secretory signal and further contains an intracellular retention sequence; (b) intracellular expression of an **antibody** encoded by said nucleic acid segment; and (c) intracellular binding of said target antigen by said **antibody**.

99. The method of claim 98, wherein the intracellular retention sequence is an endoplasmic reticulum localization sequence.

100. The method of claim 98, wherein the **antibody** is a Fab.

101. The method of claim 98 wherein the **antibody** is an **antibody** to an **HIV** envelope glycoprotein.

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(FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004

| | | | |
|---|---|---|---|
| | | E | WEINER DAVID/IN |
| L1 | 40 | S | E5 |
| L2 | 49 | S | E3 OR E5 |
| L3 | 26 | S | L2 AND (VPR OR VIRAL PROTEIN R) |
| L4 | 26 | S | L3 AND ANTIBOD? |
| | | E | LEVY DAVID/IN |
| L5 | 23 | S | E3 |
| L6 | 23 | S | L5 NOT L4 |
| | | E | REFAELI YOSEF/IN |
| L7 | 6 | S | E3 |
| L8 | 0 | S | L7 NOT (L5 OR L1) |
| | | E | MONTAGNIER LUC/IN |
| L9 | 99 | S | E3 |
| L10 | 9 | S | L9 AND (VPR OR VIRAL PROTEIN R) |
| L11 | 33171 | S | (HIV OR HUMAN IMMUNODEFICIENCY VIRUS) |

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L12           891 S L11 AND (VPR OR VIRAL PROTEIN R)
L13           548 S L12 AND ANTIBOD?
L14           156 S L13 AND (ANTIBOD?/CLM)
L15            35 S L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)
L16            26 S L15 NOT (L1 OR L9)

=> e luciw paul/in
E1              1     LUCIVERO MICHAEL/IN
E2              1     LUCIW FRED W/IN
E3              2 --> LUCIW PAUL/IN
E4              7     LUCIW PAUL A/IN
E5             10     LUCIW WILLIAM W/IN
E6              1     LUCIW WOLODYMYR/IN
E7              1     LUCK AARON JOHN/IN
E8             13     LUCK ALLAN J/IN
E9              4     LUCK ANDREAS/IN
E10             2     LUCK ARTHUR J/IN
E11             1     LUCK BILL/IN
E12             3     LUCK CHRISTOPHER F/IN

=> s e3 or e4
                2 "LUCIW PAUL"/IN
                7 "LUCIW PAUL A"/IN
L17             9 "LUCIW PAUL"/IN OR "LUCIW PAUL A"/IN

=> s l17 and (Vpr or viral protein R)
             1091 VPR
            65975 VIRAL
           174530 PROTEIN
           977413 R
               32 VIRAL PROTEIN R
                  (VIRAL(W)PROTEIN(W)R)
L18             2 L17 AND (VPR OR VIRAL PROTEIN R)

=> d l18,cbib,ab,clm,1-2

L18  ANSWER 1 OF 2  USPATFULL on STN
2001:220698 Attenuated lentivirus vectors expressing interferon.
    Yilma, Tilahun D., Davis, CA, United States
    Giavedoni, Luis D., Davis, CA, United States
    Luciw, Paul A., Davis, CA, United States
    The Regents of the University of California, Oakland, CA, United States
    (U.S. corporation)
    US 6326007 B1 20011204
    APPLICATION: US 1995-504723  19950720 (8)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      This invention discloses recombinant vectors and live attenuated
        pathogens produced by these vectors which are useful as vaccines and
        therapeutic agents. Particularly disclosed are live attenuated
        recombinant viruses that remain at very low virus loads, and preferably
        do not persist in the infected hosts. These recombinant viruses are
        useful against retroviruses such as human immunodeficiency virus and
        against acquired immunodeficiency diseases. In the recombinant vectors
        and pathogens, one or more genes, or part of the gene(s), responsible
        for pathogenesis have been completely or partially rendered
        nonfunctional, e.g., by full or partial deletion or mutagenesis.
        Further, the recombinant vectors and pathogens contain one or more genes
        encoding cytokine(s) and/or lymphokine(s).
CLM     What is claimed is:
        1. A vector, comprising: a nucleic acid molecule from a lentivirus, the
        nucleic acid molecule comprising: (a) a lentiviral 5'-LTR sequence; (b)
        a sequence encoding a cytokine; (c) a sequence encoding a lentiviral env
        protein, wherein: (i) the sequence encoding the lentiviral env protein
        comprises a sequence from a lentiviral nef gene; (ii) at least one nef
        start codon is modified or deleted; and (iii) the sequence encoding the
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lentiviral env protein is upstream of the sequence encoding the cytokine such that expression of the cytokine is enhanced compared to expression of the cytokine from a vector in which the nef start codons in the sequence encoding the lentiviral env protein have not been modified or deleted; and (d) a lentiviral 3'-LTR sequence.

2. The vector of claim 1, wherein said vector encodes an attenuated lentivirus said attenuated lentivirus expresses about $10^6$ Units of said cytokine per milliliter of cell culture about 11 days after transfection with said attenuated lentivirus in vitro.

3. The vector of claim 1, wherein said vector does not express nef.

4. The vector of claim 1, wherein said modified lentiviral nef start codon comprises an ACG sequence.

5. The vector of claim 1, said vector further comprising one or more nucleic acid molecules that encode a lentiviral protein selected from the group consisting of: tat, vis, **vpr**, and vpf.

6. The vector of claim 1, wherein said sequence encoding the lentiviral envelope protein comprises two modified lentiviral nef start codons.

7. The vector of claim 6, wherein each modified lentiviral nef start codon comprises an ACG sequence.

8. The vector of claim 1, wherein said cytokine is interferon-$\alpha$, interferon-$\beta$, or interferon-$\gamma$.

9. The vector of claim 1, wherein said cytokine is a lymphokine selected from the group consisting of: interleukin-2, and interleukin-12.

10. The vector of claim 1, wherein said lentivirus is HIV or SIV.

11. A vector, comprising: a nucleic acid molecule, said nucleic acid molecule comprising: (a) a lentiviral 5'LTR; (b) a first nucleotide sequence that encodes a lentiviral gag protein; (c) a second nucleotide sequence that encodes a lentiviral pol protein; (d) a third nucleotide sequence that encodes a lentiviral env protein, wherein the third nucleotide sequence comprises a sequence from a lentiviral nef gene and at least one nef start codon is modified or deleted, wherein said modified start codon is a sequence other than ATG, and the deleted or modified lentiviral nef start codon enhances expression of the sequence encoding the cytokine compared to the expression of a cytokine from a nucleic acid comprising an unmodified start codon; (e) a fourth nucleotide sequence encoding a cytokine, wherein the fourth nucleotide sequence is downstream from the third sequence; and (f) a lentiviral 3'-LTR.

12. The vector of claim 11, wherein said vector encodes an attenuated lentivirus said attenuated lentivirus expresses about $10^6$ Units of said cytokine per milliliter of cell culture about 11 days after transfection with said attenuated lentivirus in vitro.

13. The vector of claim 12, wherein said lentivirus is HIV or SIV.

14. The vector of claim 11, wherein the vector does not express nef.

15. The vector of claim 11, wherein said at least one more lentiviral nef start codon does not alter the amino acid sequence of said env protein.

16. The vector of claim 11, wherein said modified lentiviral nef start codon comprises an ACG sequence.

17. The vector of claim 11, said vector further comprising one or more

nucleic acid molecules that encode a lentiviral protein selected from the group consisting of tat, vif, **vpr**, and vpf.

18. The vector of claim 11, wherein said third nucleotide sequence comprises two modified lentiviral nef start codons.

19. The vector of claim 18, wherein each modified codon comprises an ACG sequence.

20. The vector of claim 11, wherein said cytokine is interferon-α, interferon-β, or interferon-γ.

21. The vector of claim 11, wherein said cytokine is a lymphokine selected from the group consisting of: interleukin-2, and interleukin-12.

L18 ANSWER 2 OF 2 USPATFULL on STN
1999:166840 Recombinant live feline immunodeficiency virus and proviral DNA vaccines.
**Luciw, Paul A.**, Davis, CA, United States
Sparger, Ellen E., Dixon, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 6004799 19991221
APPLICATION: US 1997-811828 19970305 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention discloses live-attenuated feline immunodeficiency virus (FIV), and recombinant vectors for producing them, useful as vaccines and therapeutic agents against FIV and diseases associated with virulent FIV infection. In the recombinant vectors and FIVs, one or more genes, or part of the gene(s), responsible for FIV pathogenesis have been completely or partially rendered nonfunctional, e.g., by full or partial deletion or mutagenesis. These anti-FIV vaccines may be given to susceptible hosts in the form of infectious virus or cloned DNA.
CLM What is claimed is:
1. A non-naturally occurring FIV, wherein the non-naturally occurring FIV is derived from a pathogenic FIV by specifically deleting or mutagenizing one or more of its genes or genetic elements responsible for pathogenicity, and further wherein the non-naturally occurring FIV is attenuated in pathogenicity and elicits an immune response against a pathogenic FIV in a host inoculated with the non-naturally occurring FIV.

2. The non-naturally occurring FIV of claim 1, wherein the genes or genetic element responsible for pathogenicity are selected from the group consisting of: vif, rev, OrfA/2, LTR elements, env, pol, and gag.

3. The non-naturally occurring FIV of claim 2, wherein the non-naturally occurring FIV is selected from the group consisting of: (a) a recombinant FIV with a deletion in its vif gene from about a Sau1 restriction site to about a Hind3 restriction site; (b) a recombinant FIV with about 4 to 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' and 5' LTR; (c) a recombinant FIV with a deletion in its vif gene from about a Sau1 restriction site to about a Hind3 restriction site, and with about 4 to 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' and 5' LTR; (d) a recombinant FIV with its vif gene, AP-1 and/or ATF sites in the 3' and 5' LTR deleted; and (e) a recombinant FIV with about 201 nucleotides removed from the 5' LTR and 4 or 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' LTR.

4. The non-naturally occurring FIV of claim 3, wherein the recombinant FIV is driven by an SV40pr/RU5 hybrid promoter.

5. The non-naturally occurring FIV of claim 1, wherein the FIV is

selected from the group consisting of: FIV-pPPRΔAP-1, FIV-pPPRΔATF, FIV-pPPRΔAP-1/ATF, FIV pSV-pPPRΔATF, FIV pSV-pPPRΔAP-1/ATF, FIV pPPR-pSVΔvif, and FIV-pPPRΔ4.

6. A non-naturally occurring FIV vector with one or more of its genes or genetic elements responsible for pathogenicity being specifically made either absent or fully or partially non-functional, said FIV vector being attenuated in pathogenicity; and said FIV vector preventing or delaying infection of a host by, or limiting dissemination and establishment of, a pathogenic FIV in a host inoculated with the non-naturally occurring FIV.

7. The vector of claim 6, wherein said vector is selected from the group consisting of proviral DNA, genomic RNA, and cDNA.

8. The vector of claim 7, wherein the vector is a live infectious provirus DNA.

9. The vector of claim 8, wherein the genes or genetic elements responsible for pathogenicity are selected from the group consisting of: vif, rev, OrfA/2, LTR elements, env, pol and gag.

10. The vector of claim 9, wherein the vector is a proviral DNA derived from: (a) a recombinant FIV with a deletion in its vif gene from about a Sau1 restriction site to about a Hind3 restriction site; (b) a recombinant FIV with about 4 to 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' and 5' LTR; (c) a recombinant FIV with a deletion in its vif gene from about a Sau1 restriction site to about a Hind3 restriction site, and with about 4 to 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' and 5' LTR; and (d) a recombinant FIV with its vif gene, AP-1 and/or ATF sites in the 3' and 5' LTR deleted.

11. The vector of claim 7, wherein the vector is selected from the group consisting of pPPRΔvif, pPPRΔAP-1, pPPRΔATF, pPPRΔAP-1/ATF, and pPPRΔ4.

12. A vaccine composition comprising the non-naturally occurring FIV of any of claims 1-4 or 5 in a pharmaceutically acceptable carrier, wherein the non-naturally occurring FIV is live and infectious.

13. A vaccine composition comprising the non-naturally occurring vector of any of claims 6-11, in a pharmaceutically acceptable carrier, wherein the vector is live and infectious.

14. A method for immunizing or treating an animal against infection by an FIV or its related pathogen, comprising the steps of administering to such an animal an attenuated live infectious FIV of any of claims 1-4 or 5.

15. A method for immunizing or treating an animal against infection by an FIV or its related pathogen, comprising the steps of administering the live infectious vector of any of claims 6-11 to such an animal.

16. A vector derived from the non-naturally occurring FIV of claim 1.

17. The vector of claim 16, wherein the non-naturally occurring FIV is selected from the group consisting of: FIV-pPPRΔAP-1, FIV-pPPRΔATF, FIV-pPPRΔAP-1/ATF, FIV pSV-pPPRΔATF, FIV pSV-pPPRΔAP-1/ATF, FIV pPPR-pSVΔvif, and FIV-pPPRΔ4.

18. An FIV provirus construct driven by a SV40pr/RU5 promoter.

19. An FIV virus driven by an SV40pr/RU5 promoter.

20. A method for immunizing or treating a host against FIV infection, said method consisting essentially of administering a single dose of a non-naturally occurring attenuated FIV or a non-naturally occurring FIV vector, wherein protective immunity is achieved as a result of the single dose.

21. A method for treating cats infected with FIV, said method comprising administering to said cats a non-naturally occurring attenuated FIV or a non-naturally occurring FIV vector.

22. A vaccine composition comprising a self-replicating proviral DNA construct including substantially the entire genome of an animal lentivirus with at least one mutation or deletion specifically made within a region responsible for transcription, initiation, or multiplication.

23. A vaccine composition as in claim 22, wherein the DNA construct comprises a circular DNA plasmid with a prokaryotic origin of replication.

24. A vaccine as in claim 23, wherein the deletion is in the LTR.

25. A vaccine as in claim 24, wherein the deletion is in a region selected from the group consisting of: AP1, AP4, ATF, NF-κB, C/EBP, and LBP1.

26. A method for immunizing or treating a host, comprising administering a vaccine composition of any of claims 22-25 to the host.

27. The vector of claim 8, wherein the recombinant FIV is driven by an SV40pr/RU5 hybrid promoter.

28. The non-naturally occurring FIV of claim 2 wherein the non-naturally occurring FIV is selected from the group consisting of: (a) a recombinant FIV with about 100 to 600 bases deleted or modified in its vif gene; (b) a recombinant FIV with about 30 to 300 bases deleted or modified in its rev gene; (c) a recombinant FIV with about 30 to 300 bases deleted or modified in its OrfA/2 gene; (d) a recombinant FIV with up to about 20 bases deleted from its NF-κB site; (e) a recombinant FIV with up to about 20 bases deleted from its AP-1 site; (e) a recombinant FIV with up to about 20 bases deleted from its AP-4 site; and, (f) a recombinant FIV with up to about 20 bases deleted from its ATF site.

29. The non-naturally occurring FIV of claim 2 wherein the non-naturally occurring FIV is a recombinant FIV with up to about 20 bases deleted from a site selected from the following group: NF-κB, AP-1, AP-4, and ATF.

30. The non-naturally occurring FIV of claim 29 wherein the non-naturally occurring FIV is a recombinant FIV with up to about 20 bases deleted from two or more sites selected from the following group: NF-κB, AP-1, AP-4, and ATF.

31. A non-naturally occurring FIV vector with one or more of its genes or genetic elements responsible for pathogenicity being specifically made either absent or fully or partially non-functional, said FIV vector being attenuated in pathogenicity.

32. The vector of claim 31, wherein said vector is selected from the group consisting of proviral DNA, genomic RNA, and cDNA.

33. The vector of claim 32, wherein the vector is a live infectious provirus DNA.

34. The vector of claim 31, wherein the gene or genetic element

responsible for pathogenicity is selected from the group consisting of: vif, rev, OrfA/2, LTR elements, env, pol, and gag.

35. The vector of claim 34, wherein the gene or genetic element being made fully or partially non-functional is selected from the group consisting of: (a) a vif gene with about 100 to 600 bases deleted or modified; (b) a rev gene with about 30 to 300 bases deleted or modified; (c) an Orf/A gene with about 30 to 300 bases deleted or modified; (d) a NF-κB site with up to about 20 bases deleted; (e) an AP-1 site with up to about 20 bases deleted; (e) an AP-4 site with up to about 20 bases deleted; and, (f) an ATF site with up to about 20 bases deleted.

36. The vector of claim 34, wherein the vector has up to about 20 bases deleted from two or more sites selected from the following group: NF-κB, AP-1, AP-4, and ATF.

37. The vector of claim 34, wherein the vector is a DNA derived from: (a) a recombinant FIV with a deletion in its vif gene from about a Sau1 restriction site to about a Hind3 restriction site; (b) a recombinant FIV with about 4 to 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' and 5' LTR; (c) a recombinant FIV with a deletion in its vif gene from about a Sau1 restriction site to about a Hind3 restriction site, and with about 4 to 5 nucleotides deleted from the AP-1 and/or ATF sites in the 3' and 5' LTR; and (d) a recombinant FIV with its vif gene, and AP-1 and/or ATF sites in the 3' and 5' LTR deleted.

38. The vector of claim 32, wherein the vector is selected from the group consisting of pPPRΔvif, pPPRΔAP-1, pPPRΔATF, pPPRΔAP-1/ATF, and pPPRΔ4.

```
=> d his

     (FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

     FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
                E WEINER DAVID/IN
L1            40 S E5
L2            49 S E3 OR E5
L3            26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4            26 S L3 AND ANTIBOD?
                E LEVY DAVID/IN
L5            23 S E3
L6            23 S L5 NOT L4
                E REFAELI YOSEF/IN
L7             6 S E3
L8             0 S L7 NOT (L5 OR L1)
                E MONTAGNIER LUC/IN
L9            99 S E3
L10            9 S L9 AND (VPR OR VIRAL PROTEIN R)
L11        33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L12          594 S L11 AND (VPR OR VIRAL PROTEIN R)
L13          548 S L12 AND ANTIBOD?
L14          156 S L13 AND (ANTIBOD?/CLM)
L15           35 S L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)
L16           26 S L15 NOT (L1 OR L9)
                E LUCIW PAUL/IN
L17            9 S E3 OR E4
L18            2 S L17 AND (VPR OR VIRAL PROTEIN R)

=> file wpids
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY     SESSION
FULL ESTIMATED COST                              240.03     240.24
```

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MOST RECENT DERWENT UPDATE: 200440 <200440/DW>
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http://www.stn-international.de/archive/stnews/news0104.pdf <<<

```
=> s (HIV or human immunodeficiency virus)
         17947 HIV
        146429 HUMAN
          6854 IMMUNODEFICIENCY
         35731 VIRUS
          4527 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L19       18601 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l19 and (Vpr or viral protein R)
           176 VPR
         23225 VIRAL
        115905 PROTEIN
        504718 R
             9 VIRAL PROTEIN R
                 (VIRAL(W)PROTEIN(W)R)
L20         121 L19 AND (VPR OR VIRAL PROTEIN R)

=> s l20 and antibod?
         62830 ANTIBOD?
L21         44 L20 AND ANTIBOD?

=> d l21,bib,ab,1-44

L21  ANSWER 1 OF 44  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN   2004-420517 [39]   WPIDS
DNC  C2004-157939
TI   Diagnosing HIV infection in a subject by obtaining a biological sample
     from the subject and determining the increased expression of a SHIVA in
     the biological sample.
DC   B04 D16
```

IN [illegible]
PA (SPER-I) SPERBER K
CYC 106
PI WO 2004045519 A2 20040603 (200439)* EN 164
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM
PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG UZ
VC VN YU ZA ZM ZW
ADT WO 2004045519 A2 WO 2003-US36382 20031113
PRAI US 2002-426103P 20021114
AB WO2004045519 A UPAB: 20040621
NOVELTY - Diagnosing **HIV** infection in a subject comprising:
(a) obtaining a biological sample from the subject; and
(b) determining the increased expression of a SHIVA in the biological sample.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a composition comprising an isolated polypeptide encoding a SHIVA protein or a candidate modulator of apoptosis and an immunological adjuvant, carrier or diluent;
(2) a monoclonal **antibody** that binds immunologically to a SHIVA protein;
(3) a hybridoma cell that produces a monoclonal **antibody** that binds immunologically to a SHIVA protein;
(4) a polyclonal antisera comprising **antibodies** which bind immunologically to a SHIVA protein;
(5) a nucleic acid construct comprising a polynucleotide operably linked to a heterologous promoter;
(6) altering apoptosis in a first cell;
(7) ameliorating inflammatory disease in an individual;
(8) a transgenic non-human animal having neuronal cells comprising a gene that encodes an SHIVA protein under the control of a neuron-specific promoter;
(9) a recombinant host cell transformed with an expression construct comprising the nucleic acid that encodes SHIVA under the control of a promoter;
(10) treating a subject having **HIV**-associated dementia;
(11) determining the efficacy of an **HIV** treatment regimen;
(12) screening for agents that modulate apoptosis;
(13) a kit for determining the presence of a SHIVA protein in a sample comprising the monoclonal **antibody** and a composition comprising SHIVA protein;
(14) an apoptotic protein comprising a sequence having 665 amino acids;
(15) a nucleic acid that encodes the protein; and
(16) an expression vector that comprises the nucleic acid.
ACTIVITY - Antiinflammatory; Neuroprotective.
No biological data given.
MECHANISM OF ACTION - Gene therapy.
USE - The method is useful in diagnosing **HIV** infection in a subject and in ameliorating inflammatory disease or treating a subject having **HIV**-associated dementia (claimed).
Dwg.0/21

L21 ANSWER 2 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2004-400657 [37] WPIDS
DNC C2004-150091
TI Potentiating a CD8+ or a CD4+ response to **HIV**-1 epitope in a human or treating **HIV** infection, comprises administering a nucleic acid and/or a viral vaccine encoding the **HIV**-1 epitope.
DC B04 D16
IN FRANCHINI, G; TARTAGLIA, J

CYC 103
PI WO 2004041997 A2 20040521 (200437)* EN 48
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PH PL
PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU
ZA ZM ZW
ADT WO 2004041997 A2 WO 2003-US13737 20030501
PRAI US 2002-377493P 20020501
AB WO2004041997 A UPAB: 20040611
NOVELTY - Potentiating a CD8+ or a CD4+ response to **HIV**-1 epitope in a human comprises administering at least one nucleic acid vaccine encoding at least one **HIV**-1 epitope to the human, and administering at least one viral vaccine encoding at least one **HIV**-1 epitope to the human, where the nucleic acid vaccine and the viral vaccine can be administered to the human together or in any order.
ACTIVITY - Anti-**HIV**.
No biological data given.
MECHANISM OF ACTION - Vaccine.
USE - The method is useful for maintaining an immunoprotective response in persons infected with a retrovirus (e.g. **HIV**) during early infection or after highly active anti-retroviral therapy.
Dwg.0/3

L21 ANSWER 3 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2004-169321 [16] WPIDS
DNC C2004-067054
TI New chimeric protein useful for treating **human immunodeficiency virus** infection in a subject, comprises protein transduction domain and a cytidine deaminase domain.
DC B04 D16
IN DEWHURST, S; KIM, B; SMITH, H C; SOWDEN, M P; WEDEKIND, J
PA (UYRP) UNIV ROCHESTER
CYC 105
PI WO 2004013160 A2 20040212 (200416)* EN 240
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS
LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH
PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC
VN YU ZA ZM ZW
ADT WO 2004013160 A2 WO 2003-US24458 20030805
PRAI US 2002-419982P 20021021; US 2002-401293P 20020805
AB WO2004013160 A UPAB: 20040305
NOVELTY - A chimeric protein (I) comprising a protein transduction domain and a deaminase domain, where the deaminase edits viral RNA or DNA, is a cytidine deaminase, is not APOBEC-1, does not edit ApoB1 mRNA, comprises more than two CTD-1 repeats, or comprises a CTD-1 and an anchor oligonucleotide, or the protein comprising protein transducing domain and CEM15 mimetic, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) CEM15 mimetic, where the mimetic binds viral infectivity factor;
(2) isolated nucleotide sequence (II) that encodes (I);
(3) vector (III) comprising (II);
(4) recombinant host cell comprising (III);
(5) composition (IV) comprising (I) and carrier;
(6) screening (M1) for a viral RNA deaminase mimetic involves adding the agent to be screened to a virally infected mammalian system, and detecting levels of edited viral RNA, elevated levels of edited viral RNA indicating a viral RNA deaminase mimetic;

(7) expression vector (V) comprising (II);

(8) DNA construct (VI) comprising (II), promoter sequence operably connected 5' to the DNA molecule and 3' regulatory sequence operably connected 3' of the DNA molecule;

(9) inducing (M4) somatic hypermutation in a B lymphocyte cell;

(10) inducing (M5) an immune response to an antigen in a subject

(11) expression vector comprising (VI);

(12) recombinant host cell comprising (V);

(13) recombinant host cell comprising (VI);

(14) inducing (M2) production of immunoglubulins of various classes and their subtypes;

(15) inducing (M3) class switch recombination in a B lymphocyte cell;

(16) isolated B lymphoblastic cell (VII) or other receptive cell which has taken up (I);

(17) delivery device (VIII) comprising (I); and

(18) delivery device comprising (IV).

ACTIVITY - Anti-**HIV**; Cytostatic; Anti-bacterial; Virucide; Tuberculostatic; Protozoacide; Fungicide; Immunostimulant.

The role of CEM15 deaminase activity in **HIV** infectivity suppression and the ability of Vif to suppress deaminase activity in vivo. An Env-deleted **HIV**-1 proviral DNA vector was modified by replacement of Nef with a green fluorescent protein (GFP) reporter gene and two in-frame stop codons were inserted that abolished vif production (pHR-GFP Delta Vof). Stable, HA-tagged CEM15 expressing 293T cell lines were selected with puromycin and verified by Western blotting with a HA specific monoclonal **antibody**. The addition of the epitope tag had no effect on the ability of CEM15 to suppress infectivity. Isogenic **HIV**-1 pro-viral DNAs were packaged into pseudotyped lentiviral particles by cotransfection with a plasmid encoding the vesicular stomatitis virus (VSV) G-protein into 293T cells that lack endogenous CEM15(-) or expressed wild type CEM15 (+). The resulting pseudotyped particles contained **HIV**-1 RNA of near full-length quantitated by reverse transcriptase (RT) assay. p24Gag protein content were assayed by enzyme linked immunosorbent assay (ELISA) to normalize viral particles. A defined number (1 multiply 105 cpm of RT activity) of these particles were added to target, virus susceptible MT2 cells (5 multiply 105). To assess their infectivity, the percentage of cells that expressed the GFP indicator gene encoded by the packaged recombinant **HIV**-1 genome was quantified 24 hours later by flow cytometry. The results indicated that the expression of CEM15 in 293T cells resulted in at least 100-fold decrease in Vif-viral infectivity compared to particles generated in parental 293T cells.

MECHANISM OF ACTION - Gene Therapy; Vaccine.

USE - (I) is useful for interrupting **HIV** infectivity which involves contacting an **HIV**-infected cell or a cell prior to **HIV** infection with (I) to allow delivery of (I) into the cell, where (I) binds with vif to interrupt **HIV** infectivity, for treating a subject with an **HIV** infection or at risk for an **HIV** infection which involves administering (I) to the subject,where the administration step is dose-dependent or transient. The method further comprising administering to the subject an agent that enhances the efficiency of mRNA editing function of (I). (I) is useful for treating a subject for hyper-IgM syndrome which involves administering (I) to a subject exhibiting hyper-IgM syndrome, where (I) taken up by B lymphocyte cells induces **antibody** production sufficient to treat the hyper-IgM syndrome. (I) is useful for treating a subject for B lymphocyte cell lymphoma which involves administering (I) to a subject exhibiting B lymphocyte cell lymphoma, where (I) is taken up by cancerous B lymphocyte cells, and inhibits blunt cell growth, thus treating the lymphoma. (VII) is useful for treating a subject for hyper-IgM syndrome which involves administering (VII) to a subject exhibiting hyper-IgM syndrome, where the administered B lymphocyte cells exhibit **antibody** production sufficient to treat the hyper-IgM syndrome. The method further involves prior to administering, removing the population of B lymphocyte cells from the subject and exposing the B lymphocyte cells to the chimeric protein to cause cellular uptake of the chimeric protein (all claimed).

(I) is useful for treating conditions such as cancer which include Hodgkins and non-Hodgkins lymphoma, B cell lymphoma, carcinoma, etc.,

infectious diseases which include viral diseases caused by herpes simplex virus, Hepatitis virus, Adenovirus, etc., bacterial infections caused by Mycobacterium tuberculosis, M.bovis, etc., parasitic infections caused by Toxoplasma gondii, Plasmodium falciparum, etc., fungal infections caused by Candida albicans, Cryptococcus neoformans, etc. (IV) is useful as a research tool for studying interactions between Vif and CEM15 in virions or T-cells, as targets in combinatorial chemistry protocols or other screening protocols, and as diagnostic tools related to diseases that are related to RNA or DNA editing.

DESCRIPTION OF DRAWING(S) - The figure shows schematic depiction and structure based alignment of APOBEC-1 in relation to which related proteins (ARPs).

Dwg.6/15

L21 ANSWER 4 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2004-026590 [03] WPIDS
DNC C2004-009029
TI Producing recombinant virus protein R (**Vpr**), useful in promoting immunodeficiency virus reproduction, involves transforming a host cell with an expression vector for the protein.
DC B04 D16
PA (NAKA-I) NAKAMURA T; (ORIY) ORIENTAL YEAST CO LTD
CYC 1
PI JP 2003259881 A 20030916 (200403)* 14
ADT JP 2003259881 A JP 2002-66938 20020312
PRAI JP 2002-66938 20020312
AB JP2003259881 A UPAB: 20040112

NOVELTY - Producing (M1) recombinant virus protein R (**Vpr**) protein (I) having a fully defined sequence (S1) of 96 amino acids as given in the specification, or a sequence having one or more substitutions, additions or alterations in (S1) and having biological activity, by transforming a host Escherichia coli cell (II) with an expression vector (III) containing the gene encoding (I), and culturing the transformed cell, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) the expression vector (III) useful in M1;

(2) an Escherichia coli cell (II) transformed by vector (III);

(3) recombinant virus protein R (I) produced by M1; and

(4) promoting (M2) immunodeficiency virus reproduction in a cell, tissue or organ derived from an organism by administering (I).

USE - M1 is useful for producing recombinant **Vpr** protein. (I) is useful in method M2 for promoting the reproduction of immunodeficiency virus in cell, tissue or organ derived from an organism (all claimed). (I) is useful for functional analysis of the **Vpr** protein.

ADVANTAGE - (I) is produced efficiently by (M1) at a low cost. Functional analysis showed that **Vpr** enhances reproduction of **HIV**-1 in chronic sustainable infected U1 cell. The effect of **Vpr** is neutralized by anti tumor necrosis factor (TNF) alpha **antibodies**.

DESCRIPTION OF DRAWING(S) - The figure shows the influence of **Vpr** with respect to a **HIV**-1 p24 antigen. (Drawing includes non-English language text).

Dwg.2/4

L21 ANSWER 5 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2004-010884 [01] WPIDS
CR 1999-045155 [04]; 1999-069838 [06]
DNC C2004-003093
TI New vaccine comprising an isolated DNA molecule encoding viral proteins capable stimulating an immune response against **HIV**, useful for immunizing against **HIV** infections.
DC B04 D16
IN NARAYAN, O
PA (NARA-I) NARAYAN O
CYC 1
PI US 2003220276 A1 20031127 (200401)* 48

19970502, US 2002-279992 20021024
FDT US 2003220276 A1 CIP of US 5849994
PRAI US 2002-279992 20021024; US 1995-442010 19950516;
US 1997-850492 19970502
AB US2003220276 A UPAB: 20040102
NOVELTY - A vaccine for immunizing against **HIV** comprising an isolated DNA molecule having a sequence encoding viral proteins capable of stimulating an immune response against **HIV**, where the combination of viral proteins is rendered non-pathogenic by altering the DNA molecule such that it is unable to encode at least one functional protein selected from Nef, Vpu and reverse transcriptase, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a DNA immunogenic composition derived from a viral genome coding for at least one protein capable of providing an immune response against **HIV** and having a 5' long-terminal repeat and a 3' long-terminal repeat, where the ability of the DNA immunogenic composition to integrate into a host genome has been destroyed by disruption of the 3' long-terminal repeat;

(2) a method for providing vaccination against **HIV** by administering to a recipient the DNA composition cited above;

(3) a recombinant virus comprising a DNA having the SIV LTR, gag, pol and nef genes, and **HIV**-1 env, tat, and rev genes, and a non-functional vpu gene from **HIV**-1;

(4) a DNA construct comprising SIV LTR, gag, pol and nef genes and **HIV**-1 env, tat and rev genes, and a non-functional vpu gene from **HIV**-1;

(5) an **HIV**-1/**HIV**-2 chimeric virus, where the DNA of the chimeric molecule comprises **HIV**-2 LTR gag, pol, and nef genes and **HIV**-1 env, tat and rev genes, and optionally, an **HIV**-1 vpu gene tat is rendered non-functional if present;

(6) a method for the creation of an effective vaccine for conveying immunity to **HIV**-1 virus by manipulating the **HIV**-1 virus to impede its ability to effectively replicate and/or otherwise accumulate in the infected/inoculated host; and

(7) methods for treating currently infected **HIV**-1 positive patients by administering agents that will interfere with the **HIV**-1 vpu or reverse transcriptase gene or their gene products, where such agents can be chemical, **antibody**-based or other form of bioactive molecule.

ACTIVITY - Anti-**HIV**.

No biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The vaccine is useful for immunizing against **HIV**. The compositions are useful for treating **HIV** infections or AIDS-associated illnesses.

Dwg.0/9

L21 ANSWER 6 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-851718 [79] WPIDS
CR 2002-122028 [16]; 2003-182621 [18]; 2004-021936 [02]
DNC C2003-239954
TI Enhancing the immunity of a host to infection of a first and second pathogenic virus, e.g. influenza, hepatitis, respiratory syncytial, or **HIV** infections comprises administering to the host a first and a second recombinant adenovirus.
DC B04 D16
IN WANG, D
PA (WANG-I) WANG D
CYC 1
PI US 2003138459 A1 20030724 (200379)* 185
ADT US 2003138459 A1 CIP of US 2000-585599 20000602, CIP of WO 2001-US18238 20010604, CIP of US 2001-3035 20011101, US 2003-286332 20030317
FDT US 2003138459 A1 CIP of US 6544780
PRAI US 2003-286332 20030317; US 2000-585599 20000602;
WO 2001-US18238 20010604; US 2001-3035 20011101

NOVELTY - Enhancing the immunity of a host to infection of a first and second pathogenic virus comprising administering to the host a first and a second recombinant adenovirus, is new.

DETAILED DESCRIPTION - Enhancing the immunity of a host to infection of a first and second pathogenic virus comprising administering to the host a first recombinant host cell comprising a first antigen sequence heterologous to native adenovirus and encoding a first viral antigen from the first pathogenic virus, where the expression of the first antigen by the first recombinant adenovirus elicits an immune response directed against the first viral antigen in a host upon infection of the host by the first recombinant adenovirus. The method further comprises administering to the host a second recombinant adenovirus comprising a second antigen sequence heterologous to native adenovirus and encoding a second viral antigen from the second pathogenic virus, where the expression of the second viral antigen by the second recombinant adenovirus elicits an immune response directed against the second viral antigen in a host upon infection of the host by the first recombinant adenovirus.

ACTIVITY - Virucide; Anti-**HIV**; Hepatotropic.

No biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The method is useful for enhancing immunity of the host to infections, e.g. influenza, Ebola, Marburg, Arbovirus, hepatitis, respiratory syncytial, herpes simplex or human papilloma virus, or **HIV** infections (claimed).

Dwg.0/63

L21 ANSWER 7 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-845524 [78] WPIDS
DNC C2003-237658
TI New substance specifically binds a nuclear localization signal (NLS)-containing molecule, useful for preparing a composition for treating or preventing viral infection, cell proliferation, oncogenesis or autoimmune response.
DC B04 D16
IN GRAESSMAN, A; KRICHEVSKY, A; LOYTER, A; NISSIM, A; ZAKAI, N
PA (YISS) YISSUM RES DEV CO HEBREW UNIV JERUSALEM
CYC 103
PI WO 2003089472 A2 20031030 (200378)* EN 24
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
AU 2003222419 A1 20031103 (200438)
ADT WO 2003089472 A2 WO 2003-IL328 20030421; AU 2003222419 A1 AU 2003-222419 20030421
FDT AU 2003222419 A1 Based on WO 2003089472
PRAI IL 2002-149279 20020422
AB WO2003089472 A UPAB: 20031203
NOVELTY - A new substance specifically binds a nuclear localization signal (NLS)-containing molecule, or its functional fragments or derivatives.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a composition comprising the substance and optionally diluents, additives or carriers;

(2) a vaccine comprising the substance and diluents, additives or carriers;

(3) a method of specifically inhibiting the import of a NLS-containing molecule into a nucleus of a cell;

(4) a method of inhibiting the import of **Vpr** or Tat into a nucleus of a cell;

(5) a method of inhibiting the import of the pre-integration complex (PIC) into a nucleus of a cell;

(6) a method of inhibiting viral infection;

(7) a method of inhibiting cell proliferation, oncogenesis and autoimmune response; and

(8) a method of conferring immunity against a viral infection.

ACTIVITY - Immunosuppressive; Cytostatic; Virucide. No biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The substance is useful for preparing a composition or vaccine for treating or preventing viral infection, cell proliferation, oncogenesis or autoimmune response (claimed).

Dwg.0/13

L21 ANSWER 8 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-587261 [55] WPIDS
DNC C2003-158926
TI Composition useful in the treatment of **HIV** infection comprises an **HIV** envelope antigen and a detoxified mutant A subunit of Escherichia coli heat labile toxin.
DC B04 D16
IN BARNETT, S; O'HAGAN, D; SRIVASTAVA, I; VAJDY, M
PA (CHIR) CHIRON CORP
CYC 27
PI WO 2003059385 A2 20030724 (200355)* EN 15
RW: AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT SE SI SK TR
W: CA US
ADT WO 2003059385 A2 WO 2003-US1261 20030114
PRAI US 2002-348695P 20020114
AB WO2003059385 A UPAB: 20030828

NOVELTY - A composition (C1) comprising an **HIV** envelope antigen (a) and a detoxified mutant A subunit of Escherichia coli heat labile toxin (LT) (b), is new. (LT) is LTK63 or LTR72, and (C1) optionally comprises a polynucleotide encoding either (a) or (b).

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - **HIV** vaccine; Immune response stimulator. The immune response stimulatory efficacy of a formulation comprising ogp 140 (300 micro g), gag (p24) (300 micro g) and LTK63 (100 micro g) was evaluated in rhesus macaques by intransal administration. After the course of immunization serum IgG titer were determined. Two weeks post the fifth immunization serum anti-ogp 140 IgG and anti-gag IgG titers were 4996 and 1326 respectively. The results showed that the formulation induced an **antibody**-mediated immune response.

USE - For raising an immune response (claimed); and for treating and preventing **HIV** infection.

ADVANTAGE - The composition stimulates both cell mediated and humoral immune responses.

Dwg.0/0

L21 ANSWER 9 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-577369 [54] WPIDS
DNC C2003-156169
TI Novel isolated cells that comprise transcription competent immunodeficiency virus e.g. **HIV**-1, or immunodeficiency virus-based retroviral vector integrated into its genome, useful for identifying latent **HIV** activators.
DC B04 D16
IN JORDAN, A; VERDIN, E
PA (JORD-I) JORDAN A; (VERD-I) VERDIN E; (REGC) UNIV CALIFORNIA
CYC 102
PI WO 2003054160 A2 20030703 (200354)* EN 71
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW

DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW

US 2003157693 A1 20030821 (200356)
AU 2002361797 A1 20030709 (200428)

ADT WO 2003054160 A2 WO 2002-US40698 20021218; US 2003157693 A1 Provisional US 2001-341727P 20011219, US 2002-323463 20021218; AU 2002361797 A1 AU 2002-361797 20021218

FDT AU 2002361797 A1 Based on WO 2003054160

PRAI US 2001-341727P 20011219; US 2002-323463 20021218

AB WO2003054160 A UPAB: 20030821

NOVELTY - An isolated cell (I) that comprises, integrated into the genome of the cell, a recombinant transcription-competent immunodeficiency virus-based vector, where under basal in vitro culture conditions, the immunodeficiency virus is latent, and expression of the latent immunodeficiency virus can be reactivated, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) making (I) involves introducing into population of immortalized cells in vitro a recombinant, transcription-competent **HIV** that comprises a nucleotide sequence encoding a selectable marker operably linked to a promoter, and selecting a cell population that comprises the recombinant **HIV** integrated into the genome of the cell, and that does not produce the detectable marker;

(2) an isolated immortalized cell (II) that comprises, integrated into the genome of the cell, a recombinant transcription-competent **HIV** vector that comprises a nucleotide sequence encoding a selectable marker operably linked to a promoter, where, under basal in vitro culture conditions, the **HIV** is latent, and where expression of the latent **HIV** can be reactivated; and

(3) a composition (III) comprising an agent that activates latent **HIV** identified using (II) and an excipient.

ACTIVITY - Anti-**HIV**.

No biological data is given.

MECHANISM OF ACTION - Reduces number of latently infected cells in **HIV**-infected individual; Block or reduce reactivation of latent **HIV** transcription.

USE - (II) is useful for identifying an agent that activates a latent **HIV** which involves contacting the cell with a test agent, and determining the effect, if any, of the test agent on production of the detectable marker, where production of the detectable marker indicates that the test agent activates a latent **HIV**. The detectable marker is a fluorescent protein and the determination is detection of fluorescence. (III) is useful for reducing the number of cells containing latent **HIV** in an individual, and for treating **HIV** infection in an individual which involves administering to an individual (III), and then administering to the individual an agent that inhibits an immunodeficiency virus function chosen from viral replication, viral protease activity, viral reverse transcriptase activity, viral entry into a cell, viral integrase activity, viral Rev activity, viral Tat activity, viral Nef activity, viral **Vpr** activity, viral Vpu activity, and viral Vif activity. (All claimed.) (I) is also useful for identifying agents that block or reduce reactivation of latent **HIV** transcription in response to T cell activation signals, such agents are useful to suppress activation of latent **HIV**. The agents that activate latent **HIV** are useful for reducing the reservoir of latently infected cells in an **HIV** infected individual. The agents are useful for reducing or eliminating the problem of reemergence of viremia following cessation or interruption of treatment with anti-**HIV** therapeutic agents.

ADVANTAGE - (I) is transcriptionally competent and thus is representative of cells in a reservoir of latently infected cells in an infected individual.

Dwg.0/7

L21 ANSWER 10 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2003-468412 [44] WPIDS
DNC C2003-124978
TI Identifying an agent that induces **Vpr** loss in a lentivirus-infected cell, useful for treating lentiviral infections by determining the effect of the test agent contacted with a cell that produces **Vpr** protein on the level of **Vpr** in the cell.
DC B04 D16
IN BRUNS, K; GREENE, W; HENKLEIN, P; SCHUBERT, U; SHERMAN, M; TESSMER, U; WRAY, V; GREENE, W C
PA (BRUN-I) BRUNS K; (GREE-I) GREENE W; (HENK-I) HENKLEIN P; (SCHU-I) SCHUBERT U; (SHER-I) SHERMAN M; (TESS-I) TESSMER U; (WRAY-I) WRAY V; (REGC) UNIV CALIFORNIA
CYC 101
PI WO 2003038056 A2 20030508 (200344)* EN 44
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
US 2004009909 A1 20040115 (200406)
ADT WO 2003038056 A2 WO 2002-US35110 20021031; US 2004009909 A1 Provisional US 2001-350168P 20011102, US 2002-285263 20021030
PRAI US 2001-350168P 20011102; US 2002-285263 20021030
AB WO2003038056 A UPAB: 20030710
NOVELTY - Identifying an agent that induces **Vpr** loss in a lentivirus-infected cell comprises contacting the cell that produces a **Vpr** protein with a test agent, and determining the effect, if any, of the test agent on the level of **Vpr** in the cell.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:
(1) a pharmaceutical composition comprising the agent and an excipient;
(2) inducing **Vpr** loss in a cell infected with a lentivirus by contacting the cell with the agent; and
(3) treating a lentivirus infection in an individual by administering to the individual the composition cited above, where a reduction in the level of **Vpr** in the cell treats a lentivirus infection.
ACTIVITY - Virucide; Anti-**HIV**.
No biological data given.
MECHANISM OF ACTION - Isomerase inhibitor.
USE - The methods, agents and composition are useful for treating a lentivirus infection (claimed), such as **HIV**.
Dwg.0/6

L21 ANSWER 11 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-449457 [42] WPIDS
DNN N2003-358565 DNC C2003-119425
TI Identifying compounds that inhibit **HIV Vpr** binding to apoptosis inducing factor (AIF), useful for treating cancer, inflammatory disease or autoimmune disease, comprises contacting **HIV Vpr** and AIF in the presence of a test compound.
DC B04 D16 S03
IN MUTHUMANI, K; WEINER, D B
PA (UYPE-N) UNIV PENNSYLVANIA
CYC 101
PI WO 2003040415 A1 20030515 (200342)* EN 11
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM

EP 1397520 A1 20040317 (200420) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
ADT WO 2003040415 A1 WO 2002-US16731 20020528; EP 1397520 A1 EP 2002-737214 20020528, WO 2002-US16731 20020528
FDT EP 1397520 A1 Based on WO 2003040415
PRAI US 2001-293570P 20010525
AB WO2003040415 A UPAB: 20030703
NOVELTY - Identifying compounds that inhibit **HIV Vpr** binding to apoptosis inducing factor (AIF) comprises contacting **HIV Vpr** or its fragment known to interact with AIF, and AIF or its fragment that interacts with **Vpr** in the presence of a test compound, and comparing the level of **HIV Vpr** binding to AIF to the level of **HIV Vpr** binding to AIF in the absence of the test compound.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a kit for performing the method cited above, comprising:

(a) a first container comprising **HIV Vpr** or its fragment known to interact with apoptosis inducing factor (AIF);

(b) a second container comprising AIF or its fragment which interacts with **Vpr** in the presence of a test compound; and

(c) optionally, instructions for performing the test assay; and

(2) identifying compounds that inhibit **HIV Vpr**/AIF nuclear translocation comprises contacting, in the presence of a test compound, cells comprising **HIV Vpr** or its fragment known to interact with AIF and AIF or its fragment which interacts with **Vpr**, and comparing the level of **Vpr**/AIF in the nucleus and/or cytoplasm to the level of **Vpr**/AIF in the nucleus and/or cytoplasm in the absence of the test compound.

ACTIVITY - Antiinflammatory; Cytostatic; Anti-**HIV**; Immunosuppressive. No biological data given.

MECHANISM OF ACTION - Apoptosis Inhibitor; Apoptosis Stimulator.

USE - The methods are useful for identifying compounds that inhibit **HIV Vpr** binding to apoptosis inducing factor (AIF) or **HIV Vpr**/AIF nuclear translocation. The methods are useful for drug screening assays. The compounds or inhibitors are useful for preventing or inducing apoptosis used in treating inflammatory disease, autoimmune disease or cancer, or as anti-**HIV** agents.

Dwg.0/0

L21 ANSWER 12 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-438926 [41] WPIDS
DNC C2003-116141
TI Novel DNA or RNA construct for increasing immune response of warm-blooded animal, has **Vpr** activated promoter, DNA segment encoding interleukin 2 and secretory DNA encoding signal peptide functional in mammary cells.
DC B04 D16
IN ALFIERI, C; ROUX, P; TANNER, J
PA (ALFI-I) ALFIERI C; (ROUX-I) ROUX P; (TANN-I) TANNER J
CYC 1
PI US 2003017137 A1 20030123 (200341)* 28
ADT US 2003017137 A1 US 1998-120286 19980722
PRAI US 1998-120286 19980722
AB US2003017137 A UPAB: 20030630
NOVELTY - A DNA or RNA construct (I) capable of expressing interleukin (IL)-2 in a warm-blooded animal or biological preparation, comprising a **Vpr** activated promoter, a transcribable DNA segment coding for IL-2, and a secretory DNA encoding for a signal peptide functional in mammary cells and operably linked between the promoter and the DNA segment to facilitate secretion of IL-2.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) inhibiting (M1) expression of IL-8 of a warm-blooded animal or a biological preparation, by administering a pharmaceutically effective amount of a **Vpr** inhibitor;

(3) Stimulating IL-5 expression in a mammal in need of the expression, by administering a pharmaceutically effective amount of a pharmaceutically acceptable formulation comprising a **Vpr** protein to the mammal; and

(3) determining the interaction between **Vpr** and other proteins, by co-precipitation of **Vpr** and associated cellular proteins using anti-**Vpr antibody** followed by protein gel electrophoresis, development of a yeast two hybrid system in which a **Vpr**-Gal4 construct is introduced into yeast to screen human cDNAs expressed in yeast library and detection of Gal4 insensitive colonies, and constriction of **Vpr** deletion mutants to identify both association of cellular proteins with **Vpr** or **Vpr** subdomains.

ACTIVITY - Cytostatic; Anti-**HIV**.

MECHANISM OF ACTION - Gene therapy; Stimulator of immune response.

No biological data given.

USE - (I) is useful for increasing the immune response of a warm-blooded animal or biological preparation, by introducing (I) in stem cells, antigen presenting cells or immune cell leukocytes, fibroblasts and epithelial cells, of the warm-blooded animal or biological preparation to obtain a transfected cell populations, and administering a pharmaceutically effective amount of the transfected cell populations to the warm-blooded animal or biological preparation. The warm-blooded animal is an immunocompromised patient (claimed).

The method is useful for stimulating immune response in immunocompromised patients affected with **HIV**, cancer and other immunocompromised patients.

Dwg.0/5

L21 ANSWER 13 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-430468 [40] WPIDS
DNC C2003-113870
TI Novel synthetic therapeutic compounds that cross-react with epitopes of **HIV Vpr** proteins, useful for inhibiting **HIV** proliferation and propagation and treating AIDS.
DC B04 D16
IN NICOLETTE, C A; WALKER, B D
PA (NICO-I) NICOLETTE C A; (WALK-I) WALKER B D; (GENZ) GENZYME CORP; (MASS-N) MASSACHUSETTS GEN HOSPITAL
CYC 100
PI WO 2003037264 A2 20030508 (200340)* EN 65
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
US 2003165517 A1 20030904 (200359)
ADT WO 2003037264 A2 WO 2002-US34688 20021029; US 2003165517 A1 Provisional US 2001-345957P 20011029, US 2002-283618 20021029
PRAI US 2001-345957P 20011029; US 2002-283618 20021029
AB WO2003037264 A UPAB: 20030624
NOVELTY - Synthetic therapeutic compounds (I) that specifically cross-react with the epitope spanning amino acids 59-67 of **Vpr** which has been isolated from one species of native **HIV Vpr** protein, are new.

DETAILED DESCRIPTION - Synthetic therapeutic compounds (I) that specifically cross-reacts with the epitope spanning amino acids 59-67 of **Vpr** which has been isolated from one species of native **HIV Vpr** protein, and comprises a sequence (S) chosen from (S1-S6):

Phe-Leu-Tyr-Glu-Gln-Gly-Met- Phe-Val (S1)
Phe-Leu-Tyr-Glu-Gln-Gly-Ile-Phe-Val (S2)
Phe-Leu-Lys-Met-Trp-Lys-Asp-Ala-Val (S3)
Phe-Leu-Ser-Trp-Thr-Leu-Pro- Arg-Val (S4)
Phe-Leu-Gly-Gly-His-Trp-Gly-Thr-Val (S5)
Phe-Leu-Trp-Trp- Phe-Thr-Ser-Thr-Val (S6)

Independent claims are also included for:

(1) a peptide (II) comprising a sequence of 78 amino acids given in the specification, where amino acids 59-67 are Phe, Leu, Tyr/Lys/Ser, Glu/Met/Trp, Gln/Trp/Thr, Gly/Lys/Leu, Met/Ile/Asp/Pro, Phe/Ala/Arg and Val, respectively;

(2) a polynucleotide that encodes (II);

(3) a polynucleotide that encodes (I);

(4) an **antibody** (III) that recognizes and binds (I);

(5) an immune effector cell (IV) that has been raised in vivo or in vitro in the presence and at the expense of an antigen presenting cell that presents (I), in the context of an MHC molecule;

(6) a composition (V) comprising one or more ligand which is individually characterized by an ability to elicit an immune response against the same native ligand, where the ligand is chosen from (S);

(7) a host cell comprising at least one or more ligands, where the ligands are individually characterized by an ability to elicit an immune response against the same native ligand, where the ligand is chosen from (S); and

(8) a composition comprising the above host cell.

ACTIVITY - Anti-**HIV**.

No biological data given.

MECHANISM OF ACTION - Vaccine; Inhibitor of **HIV** replication and propagation.

USE - (I) is useful for inducing an immune response in a subject, where the compound is delivered in the context of an major histocompatibility complex (MHC) molecule, where the molecule presents the compound on the surface of an antigen presenting cell.

(III) is useful for immunotherapy.

(IV) is useful for adoptive immunotherapy.

(V) is useful for inducing an immune response in a subject (claimed).

(I) is useful for generating **antibodies** that specifically recognize and bind to these molecules. (I) is useful for inducing an immune response in a subject having an **HIV** infection and in particular, **HIV**-1, **HIV**-2 or related virus SIV. (I) is useful for preparing medicaments for diagnosis and treatment of diseases such as AIDS and AIDS-related complex (ARC).

Dwg.0/0

L21 ANSWER 14 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-373754 [36] WPIDS
DNC C2003-099535
TI Use of biologically active **HIV** Tat or tat DNA for activating or targeting antigen presenting cells, or as a vaccine for preventing or treating e.g. **HIV**/AIDS, hepatitis, tumors (Kaposi's sarcoma), or inflammations (Crohn's disease).
DC B04 C06 D16
IN ENSOLI, B
PA (SUPE-N) INST SUPERIORE DI SANITA
CYC 101
PI EP 1279404 A1 20030129 (200336)* EN 98
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
WO 2003009867 A1 20030206 (200336) EN
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
EP 1425035 A1 20040609 (200438) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
ADT EP 1279404 A1 EP 2001-118114 20010726; WO 2003009867 A1 WO 2002-EP8377 20020726; EP 1425035 A1 EP 2002-767265 20020726, WO 2002-EP8377 20020726

PRAI EP 2001-118114 20010726
AB EP 1279404 A UPAB: 20030609
NOVELTY - Use of **HIV**-1 Tat, tat DNA, or their fragments or derivatives, for:

(a) targeting, activating, enhancing, or delivering antigen-presenting cells;

(b) identifying macrophages, dendritic or activated endothelial cells for diagnosis of **HIV**/AIDS, inflammatory, angiogenic diseases, or tumors;

(c) delivering therapeutics intracellularly or to the cell membrane; or

(d) producing a vaccine for the diseases cited in (b).

DETAILED DESCRIPTION - Use of **HIV**-1 Tat, tat DNA, or their fragments or derivatives for:

(a) targeting, activating or enhancing, or delivering in vitro and in vivo antigen-presenting cells;

(b) identifying dendritic cells, macrophages or activated endothelial cells for diagnostic purposes in **HIV**/AIDS or other infectious diseases, inflammatory or angiogenic diseases, or tumors;

(c) delivering therapeutic compounds intracellularly or to the cells membrane; or

(d) manufacturing a vaccine or medicament for the diseases cited in (b).

INDEPENDENT CLAIMS are also included for the following:

(1) A pharmaceutical composition comprising the biologically active **HIV** Tat or tat DNA, or their fragments of derivatives, combined or fused with at least one of the following: antigens, therapeutic compounds, adjuvants or support particles; and

(2) A kit for targeting in vivo or in vitro antigen-presenting cells, or for scopes of identifying, isolating or purifying such cells in blood and tissues from pathological or physiological conditions (e.g. infections, inflammation, angiogenesis or tumors); where the kit comprises the Tat module combined with or fused to a tag that can be detected by means of its reactive properties.

ACTIVITY - Antibacterial; Anti-**HIV**; Protozoacide; Virucide; Cytostatic; Antiinflammatory; Immunosuppressive; Dermatological; Antirheumatic; Antiarthritic; Antithyroid; Antiallergic; Antidiabetic; Antiarteriosclerotic; Immunostimulant.

MECHANISM OF ACTION - Chemokine Modulator; Cytokine Modulator; Vaccine.

The production of the cytokines interleukin-12 (IL-12) and tumor necrosis factor alpha (TNF- alpha ), known to activate immune cells and to induce Th-1 type responses, and of the beta -chemokine RANTES, MIP-1 alpha and MIP-1 beta , which are known mediators of immune responses, were assessed by ELISA in the supernatants of cells cultured (18 hours) with Tat protein, reconstitution buffer (negative control) or LPS (positive control). Results showed that tat increased the levels of IL-12 (23-fold, p less than 0.02) and TNF- alpha (20-fold, p less than 0.03) as compared to cells treated with buffer alone. Similarly, Tat enhanced the production of RANTES (10-fold, p less than 0.02), MIP-1 alpha (97-fold, p less than 0.005) and MIP-1 beta (15-fold, p less than 0.01). The negative control had no effect, while the positive control markedly enhanced the production of both cytokines and beta -chemokines.

USE - The **HIV** Tat or tat DNA is useful for targeting (in vivo or in vitro) antigen-presenting cells (APCs), or as an adjuvant for activating or enhancing antigen-presenting function of cells, particularly to induce Th-1 type immune response against **HIV**/AIDS, other infectious diseases or tumors. The **HIV** Tat or tat DNA is also useful for delivering antigen(s) to APCs to induce an immune response. In particular, the **HIV** Tat or tat DNA is useful as a vaccine for preventing or treating **HIV**/AIDS or other infectious diseases (e.g. sexual infectious diseases, endocarditis, urinary tract infection, osteomyelitis, cutaneous infections, streptococcus or staphylococcus infections, pneumococcal infections, tetanus, meningococcus infections, malaria, syphilis, herpes, hepatitis, papilloma virus infections, influenza, lysteria or vibrio cholera), tumors (e.g. Kaposi's sarcoma, sarcomas, chronic leukemia, or neoplasias of the

skin, lymphatic cells, breast, lungs, CNS, or inflammatory (e.g. systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, dermatomiositis, Sjogren syndrome, Goodpasture syndrome, arthritis, Crohn's disease, thyroiditis, scleroderma, or allergies) or angiogenic diseases (e.g. diabetic retinopathy, atherosclerosis, excessive wound repair, colon angiodisplasia, angioedema or angiofibromas) (all claimed). Dwg.0/18

L21 ANSWER 15 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-316488 [31] WPIDS
CR 2004-191274 [18]
DNN N2003-252044 DNC C2003-083248
TI New peptide that binds to protein phosphatase holoenzyme, and that is a fragment of a viral, parasite or cellular protein, useful e.g. for treating cancer, **human immunodeficiency virus** (**HIV**) and malaria.
DC B04 D13 D16 J04 S03
IN CAYLA, X; GARCIA, A; LANGSLEY, G; REBOLLO, A
PA (CNRS) CNRS CENT NAT RECH SCI; (CNSJ) CONSEJO SUPERIOR INVESTIGACIONES CIENTIF; (INRG) INRA INST NAT RECH AGRONOMIQUE; (INSP) INST PASTEUR; (CNRS) CENT NAT RECH SCI
CYC 100
PI FR 2827866 A1 20030131 (200331)* 51
WO 2003011898 A2 20030213 (200331) FR
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
ADT FR 2827866 A1 FR 2001-10139 20010727; WO 2003011898 A2 WO 2002-FR2705 20020726
PRAI FR 2001-10139 20010727
AB FR 2827866 A UPAB: 20040316
NOVELTY - A peptide (I), shorter than 30, preferably 20, amino acids long that binds specifically, in vitro, to a type 2A protein phosphatase holoenzyme (II), is new.

DETAILED DESCRIPTION - A new peptide (I), is shorter than 30, preferably 20, amino acids (aa) in length and binds specifically, in vitro, to a type 2A protein phosphatase (PP2A) holoenzyme (II). (I) is:

(i) a fragment of a viral, parasite or cellular protein, i.e. t antigen of simian virus (SV)40 or polyoma, a medium t antigen of polyoma, a subunit of type B (Bm B' or B'') of PP2A, CK alpha 2, CaMIV, p70S6 kinase, Pak1/Pak3, Tap42/ alpha 4, PTPA, Set/I1/I2-PPA2, E4orf4, tau, cluster of differentiation (CD)28 or **Vpr**; or

(ii) a variant of (i) having aa deleted or substituted provided it retains ability to bind to (II) or one of its subunits.

INDEPENDENT CLAIMS are also included for the following:

(1) polypeptides comprising repeats of (I);

(2) polynucleotides (III) that encode (I);

(3) an expression vector containing (III) and expression control elements;

(4) purified mono- or poly-clonal **antibodies** (Ab) that can bind specifically to (I);

(5) identifying (I); and

(6) producing (I).

ACTIVITY - Anti-**HIV**; Virucide; Protozoacide; Cytostatic; Nootropic; Neuroprotective; Apoptotic. No biological data is given.

MECHANISM OF ACTION - Interaction between the protein from which (I) is derived and (II), or its subunits competitive inhibitor; Enzyme inhibitor; Gene therapy.

USE - (I) Derived from the **Vpr** protein of **human immunodeficiency virus** (**HIV**) is used to treat tumors and (I) derived from parasites is used to inhibit parasite development, specifically for treating **HIV** infection or malaria. Polynucleotides (III) that encode

(I), expression vectors containing (III), and antibodies (IV), specific for (I) are used similarly. Ab are also used for in vitro diagnosis of parasitic and viral infections. Also, where (I) is derived from tau protein it is used for treating Alzheimer's disease.

ADVANTAGE - Unlike the parent protein, (I) is easy to prepare, in high yield and low cost. Also it is more stable and easily transfered to the cytoplasm or nuclei of cells.

Dwg.0/2

L21 ANSWER 16 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-278761 [27] WPIDS
CR 2003-221593 [21]; 2003-221602 [21]
DNC C2003-073023
TI New expression cassettes and polynucleotides encoding **HIV** Gag, Nef, Prot, Tat, Rev, Vif, **Vpr**, Vpu, or Env polypeptides, useful for DNA immunization or generating an immune response against **HIV** in a subject.
DC B04 D16
IN BARNETT, S; LIAN, Y; ZUR MEGEDE, J Z; MEGEDE, J Z; BARNETT, S W; ZUR MEGEDE, J
PA (BARN-I) BARNETT S; (LIAN-I) LIAN Y; (ZMEG-I) ZUR MEGEDE J Z; (MEGE-I) MEGEDE J Z; (CHIR) CHIRON CORP
CYC 89
PI WO 2003020876 A2 20030313 (200327)* EN 214
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
US 2003170614 A1 20030911 (200367)
US 2003194800 A1 20031016 (200369)
EP 1427806 A2 20040616 (200439) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
ADT WO 2003020876 A2 WO 2002-US21342 20020705; US 2003170614 A1 Provisional US 2001-316860P 20010831, Provisional US 2002-349728P 20020116, CIP of US 2002-190434 20020705, US 2002-241009 20020906; US 2003194800 A1 Provisional US 2001-316860P 20010831, Provisional US 2002-349728P 20020116, US 2002-190434 20020705; EP 1427806 A2 EP 2002-746891 20020705, WO 2002-US21342 20020705
FDT EP 1427806 A2 Based on WO 2003020876
PRAI US 2002-349728P 20020116; US 2001-316860P 20010831;
US 2002-190434 20020705; US 2002-241009 20020906
AB WO2003020876 A UPAB: 20040621
NOVELTY - An expression cassette comprising a polynucleotide sequence encoding a polypeptide including an **HIV** Gag, Nef, Prot, Tat, Rev, Vif, **Vpr**, Vpu, or Env polypeptide, is new.

DETAILED DESCRIPTION - The polynucleotide sequence encoding:

(a) the Gag polypeptide comprises a sequence having at least 90% sequence identity to any one of 5 sequences of 4773-5274 base pairs (bp), a sequence having at least 98% sequence identity to any one of 4 sequences (e.g. 4883 bp), or a sequence having at least 95% sequence identity to a sequence of 3004 bp, all fully defined in the specification;

(b) the Nef polypeptide comprises a sequence having at least 90% sequence identity to any one of 6 fully defined sequences of 570-3735 bp given in the specification;

(c) the Prot polypeptide comprises a sequence having at least 98% sequence identity to a fully defined sequence of 2262 bp given in the specification;

(d) the Tat polypeptide comprises a sequence having at least 90% sequence identity to any one of 8 fully defined sequences of 1281-5283 bp given in the specification;

(e) the Rev polypeptide comprises a sequence having at least 90% sequence identity to a fully defined sequence of 348 bp given in the specification;

(f) the Tat polypeptide comprises a sequence having at least 90% sequence identity to at least 30 contiguous base pairs of a fully defined sequence of 576 bp given in the specification;

(g) the **Vpr** polypeptide comprises a sequence having at least 90% sequence identity to at least 20 contiguous base pairs of a fully defined sequence of 291 bp given in the specification;

(h) the Vpu polypeptide comprises a sequence having at least 90% sequence identity to at least 20 contiguous base pairs of a fully defined sequence of 243 bp given in the specification; or

(i) the Env polypeptide comprises a sequence having at least 90% sequence identity to any one of 4 fully defined sequences each comprising 2007 bp given in the specification.

INDEPENDENT CLAIMS are included for the following:

(1) a recombinant expression system for use in a selected host cell comprising any one of the expression cassettes cited above, where the polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell;

(2) a cell comprising the expression cassette, where the polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell;

(3) a method for producing a polypeptide including **HIV** Gag polypeptide sequences, comprising incubating the cells of (2) to produce the polypeptide;

(4) a gene delivery vector for use in a mammalian subject, where the vector comprises the expression cassette, and where the polynucleotide sequence is operably linked to control elements compatible with expression in the subject;

(5) a method of DNA immunization of a subject comprising introducing the gene delivery vector into the subject under conditions that are compatible with expression of the expression cassette in the subject; and

(6) a method of generating an immune response in a subject comprising transfecting cells of the subject with the gene delivery vector under conditions that permit the expression of the polynucleotide and production of the polypeptide to elicit an immunological response to the polypeptide.

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - Vaccine. Rabbits were immunized intramuscularly, mucosally or intradermally with plasmid DNAs encoding the **HIV** proteins. The nucleic acid immunizations were followed by protein boosting after the initial immunization. Constructs comprising the synthetic **HIV** polypeptide-encoding polynucleotides were highly immunogenic and generated substantial antigen binding **antibody** responses after only two immunizations.

USE - The expression cassettes, **HIV** polypeptides and polynucleotides encoding the **HIV** polypeptides are useful for DNA immunization or generating an immune response against **HIV** in a subject. The polynucleotides are also useful for generating packaging cell lines or producing the **HIV** polypeptides.

Dwg.0/68

L21 ANSWER 17 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-239474 [23] WPIDS
DNC C2003-061570
TI Use of an **HIV** Tat, Nef or gp120 protein or polynucleotide in manufacturing a vaccine for a prime-boost delivery for the prophylactic or therapeutic immunization of humans against **HIV**.
DC B04 D16
IN ERTL, P F; TITE, J P; VAN WELY, C A; VOSS, G
PA (GLAX) GLAXO GROUP LTD; (GLAX) GLAXOSMITHKLINE BIOLOGICALS SA
CYC 101
PI WO 2003011334 A1 20030213 (200323)* EN 108
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT

ZW
EP 1411979 A1 20040428 (200429) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
ADT WO 2003011334 A1 WO 2002-EP8343 20020726; EP 1411979 A1 EP 2002-791473 20020726, WO 2002-EP8343 20020726
FDT EP 1411979 A1 Based on WO 2003011334
PRAI GB 2001-18367 20010727
AB WO2003011334 A UPAB: 20030407

NOVELTY - Use of an **HIV** Tat protein or polynucleotide, an **HIV** Nef protein or polynucleotide, or an **HIV** Tat protein or polynucleotide linked to an **HIV** Nef protein or polynucleotide, and an **HIV** gp120 protein or polynucleotide in manufacturing a vaccine for a prime-boost delivery for the prophylactic or therapeutic immunization of humans against **HIV**.

DETAILED DESCRIPTION - Use of an **HIV** Tat protein or polynucleotide, an **HIV** Nef protein or polynucleotide, or an **HIV** Tat protein or polynucleotide linked to an **HIV** Nef protein or polynucleotide, and an **HIV** gp120 protein or polynucleotide in manufacturing a vaccine for a prime-boost delivery for the prophylactic or therapeutic immunization of humans against **HIV**. The protein or polynucleotide is delivered via a bombardment approach.

INDEPENDENT CLAIMS are included for the following:

(1) a recombinant DNA molecule comprising a Nef and/or Tat and/or gp120 gene in a vector in which the gene of interest is inserted 3' to an enhanced HCMV IE1 promoter;

(2) particles, preferably gold particles, coated with recombinant DNA comprising a Nef and/or Tat and/or gp120 gene in a vector; and

(3) a kit comprising at least two different vaccine compositions including:

(a) a composition comprising particles coated with DNA encoding gp120 and nef and/or tat or neftat; and

(b) a composition comprising gp120 and nef and/or tat or nef-tat DNA or proteins, where the DNA or proteins are not coated onto the particles.

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - Vaccine.

Groups of 4 rhesus monkeys were immunized intramuscularly at 0, 1 and 3 months with the following vaccine compositions:

(a) Group 1 - adjuvant 2 and gp120;

(b) Group 2 - adjuvant 2, gp120, Nef-Tat and (simian immunodeficiency virus) SIV Nef;

(c) Group 3 - adjuvant 2, Nef-Tat and SIV Nef;

(d) Group 4 - adjuvant 6, gp120, Nef-Tat and SIV Nef;

(e) Group 5 - adjuvant 2, Nef-Tat and SIV Nef; and

(f) Group 6 - adjuvant 2.

Adjuvant 2 comprises squalene/tocopherol/Tween 80/3D-MPL/QS21, while adjuvant 6 comprises alum and CpG. One month after the last immunization, all animals were challenged with a pathogenic (human-simian immunodeficiency virus) SHIV (strain 89.6p). From the week of challenge (week 16) blood samples were taken periodically at the indicated time points to determine the percent of CD4-positive cells among peripheral blood mononuclear cells by fluorescent activated cell sorting (FACS) analysis and the concentration of RNA viral genomes in the plasma by cDNA assay. CD4-positive cells decline after challenge in all animals of groups 1, 3, 5 and 6 except one animal in each of groups 1 and 6 (control group).

All animals in group 2 exhibit a slight decrease in CD4-positive cells and recover to baseline levels over time. A similar trend is observed in group 4 animals. Virus load data are almost the inverse of CD4 data. Virus load declines below the level of detection in three-quarters of group 2 animals (and in one control animal that maintains its CD4-positive cells), and the fourth animal shows only marginal virus load. Most of the other animals maintain a high or intermediate virus load. Anti-Tat and anti-Nef **antibody** titers measured by ELISA were 2-3-fold higher in group 3 than in group 5 throughout the course of the study. At week 68 (56 weeks post-challenge) all animals from the groups that had

received the full antigen combination (groups 2 and 4) were still alive, while most of the animals in the other groups had to be euthanized due to AIDS-like symptoms.

USE - The **HIV** Tat protein or polynucleotide, **HIV** Nef protein or polynucleotide, or **HIV** Tat protein or polynucleotide linked to an **HIV** Nef protein or polynucleotide, and **HIV** gp120 protein or polynucleotide are useful in manufacturing a vaccine for a prime-boost delivery for the prophylactic or therapeutic immunization of humans against **HIV**. The recombinant DNA molecule is also useful in manufacturing an **HIV** vaccine for the prophylactic or therapeutic immunization of humans (all claimed). Dwg.0/26

L21 ANSWER 18 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-221602 [21] WPIDS
CR 2003-221593 [21]; 2003-278761 [27]
DNC C2003-056373
TI New synthetic polynucleotides encoding antigenic **HIV** type B and/or type C polypeptides, useful as immunogenic compositions or vaccines for generating humoral or cellular immune responses against **HIV** in a subject, especially humans.
DC B04 C06 D16
IN BARNETT, S; LIAN, Y; MEGEDE, J Z; BARNETT, S W; ZUR MEGEDE, J
PA (BARN-I) BARNETT S; (LIAN-I) LIAN Y; (MEGE-I) MEGEDE J Z; (CHIR) CHIRON CORP
CYC 89
PI WO 2003004657 A1 20030116 (200321)* EN 256
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
US 2003198621 A1 20031023 (200370)
EP 1409694 A1 20040421 (200427) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
ADT WO 2003004657 A1 WO 2002-US21421 20020705; US 2003198621 A1 Provisional US 2001-303192P 20010705, Provisional US 2001-316860P 20010831, Provisional US 2002-349728P 20020116, Provisional US 2002-349793P 20020116, Provisional US 2002-349871P 20020116, US 2002-190305 20020705; EP 1409694 A1 EP 2002-749827 20020705, WO 2002-US21421 20020705
FDT EP 1409694 A1 Based on WO 2003004657
PRAI US 2002-349871P 20020116; US 2001-303192P 20010705;
US 2001-316860P 20010831; US 2002-349728P 20020116;
US 2002-349793P 20020116; US 2002-190305 20020705
AB WO2003004657 A UPAB: 20040426
NOVELTY - A synthetic polynucleotide encoding 2 or more immunogenic **HIV** polypeptides, where at least 2 of the polypeptides are derived from different **HIV** subtypes, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) an expression cassette comprising the synthetic polynucleotide;

(2) a recombinant expression system for use in a host cell comprising the expression cassette, where the polynucleotide sequence is operably linked to control elements compatible with expression in the host cell;

(3) a cell comprising the expression cassette, where the polynucleotide sequence is operably linked to control elements compatible with expression in the cell;

(4) a gene delivery vector for use in a mammalian subject, where the vector comprises the expression cassette, and the polynucleotide sequence is operably linked to control elements compatible with expression in the subject;

(5) a method for producing a polypeptide including 2 or more **HIV** polypeptides from different subtypes by incubating the cells of (3) to produce the polypeptide;

(6) a method of DNA immunization of a subject by introducing the gene

delivery vector into a subject under conditions that are compatible with expression of the expression cassette in the subject; and

(7) a method of generating an immune response in a subject by transfecting the cells of the subject with the gene delivery vector, under conditions that permit the expression of the polynucleotide and the production of the polypeptide, thus eliciting an immunological response to the polypeptide.

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - Vaccine; Gene Therapy.

No biological data given.

USE - The polynucleotide is useful for immunization, generation of packaging cell lines, or production of **HIV** polypeptides. The polynucleotide and its encoded proteins are useful as immunogenic compositions or vaccines for generating humoral or cellular immune responses against **HIV** in a subject, or for inducing neutralizing **antibodies** against **HIV**. The gene delivery vector comprising the polynucleotide is also useful for DNA immunization of, or for generating an immune response (e.g. a humoral or cellular immune response) in, a subject such as a mammal, particularly a human (claimed).

Dwg.0/96

L21 ANSWER 19 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-210119 [20] WPIDS
DNC C2003-053503
TI Bacteriophage preparation for stimulating death of infected cell, has bacteriophage that enters cell and lyses pathogenic bacteria that infect cells, linked to annihilation moiety that stimulates death of infected cells.
DC B04 C03 D16
IN PASECHNIK, V; WEST, D
PA (POLY-I) POLYANSKAYA N; (REGM-N) REGMA BIO TECHNOLOGIES LTD
CYC 100
PI WO 2003000274 A2 20030103 (200320)* EN 45
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
ADT WO 2003000274 A2 WO 2002-GB2879 20020621
PRAI GB 2001-15385 20010622
AB WO2003000274 A UPAB: 20030324

NOVELTY - A bacteriophage preparation (I), comprises a bacteriophage (II) adapted to enter eukaryotic cell and/or cellular compartment (III), where (II) is lytic to pathogenic bacterial strain (PB) that infect (III), and (III) is fused, linked or adapted to express an annihilation moiety (AM), where AM is adapted to cause or stimulate death or inactivation of cell in the presence of PB.

DETAILED DESCRIPTION - A bacteriophage preparation (I),comprises a bacteriophage (II) which is adapted to enter a eukaryotic cell and/or cellular compartment (III), which (II) is lytic towards at least one strain of pathogenic bacteria (PB) which may infect (III), where (II) is fused to, linked to or is adapted to express an annihilation moiety (AM), where AM is adapted when in the presence of at least one specific pathogen to cause or stimulate the death or inactivation of the cell, such that (I) is capable of causing or stimulating the death or inactivation of cells which are infected with the pathogen.

ACTIVITY - Tuberculostatic; Anti-**HIV**; Virucide; Protozoacide; Antibacterial.

No supporting data is given.

MECHANISM OF ACTION - Stimulates death or inactivation of cells infected with a pathogen (claimed).

No supporting data is given.

USE - (I) is useful for producing the death or inactivation of a cell

infected by a pathogen. (1) is also useful in the manufacture of a pharmaceutical composition for administration to a patient in need of it, and in the manufacture of a pharmaceutical composition for use in the treatment and/or prophylaxis of a disease which is mediated or characterized by intracellular infection by a pathogen, including in particular tuberculosis, acquired immunodeficiency syndrome (AIDS), **human immunodeficiency virus** (**HIV**) infection, and malaria (claimed).

ADVANTAGE - The pharmaceutical compositions are capable of targeting intracellular infection without an adverse effect upon uninfected host cells, and are capable of effectively combating pathogen strains including multidrug resistant and non-multidrug resistant strains. The bacteriophage preparation is capable of achieving the destruction of both the pathogen in question and its host, hence bringing about rapid elimination of a systemic infection.

Dwg.0/5

L21 ANSWER 20 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-140646 [13] WPIDS
DNN N2003-111649 DNC C2003-035844
TI Identifying whether a compound inhibits entry of a virus into a cell for determining susceptibility and resistance of a virus to the compound, by using recombinant virus assays.
DC B04 D16 S03
IN HUANG, W; PARKIN, N T; PETROPOULOS, C J; WHITCOMB, J M; WHITCOMB, J
PA (VIRO-N) VIROLOGIC INC; (HUAN-I) HUANG W; (PARK-I) PARKIN N T; (PETR-I) PETROPOULOS C J; (WHIT-I) WHITCOMB J M; (WHIT-I) WHITCOMB J
CYC 26
PI WO 2002099383 A2 20021212 (200313)* EN 143
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: BR CN JP SG ZA
US 2002182592 A1 20021205 (200313)
EP 1402076 A2 20040331 (200424) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
US 2004110125 A1 20040610 (200438)
ADT WO 2002099383 A2 WO 2002-US18078 20020604; US 2002182592 A1 US 2001-874475 20010604; EP 1402076 A2 EP 2002-756136 20020604, WO 2002-US18078 20020604; US 2004110125 A1 Provisional US 2001-295871P 20010604, US 2002-164290 20020604
FDT EP 1402076 A2 Based on WO 2002099383
PRAI US 2001-874475 20010604; US 2001-295871P 20010604;
US 2002-164290 20020604
AB WO 200299383 A UPAB: 20040102
NOVELTY - Identifying whether a compound inhibits entry of a virus into a cell for determining susceptibility and resistance of a virus to the compound, is new.

DETAILED DESCRIPTION - Identifying whether a compound inhibits entry of a virus into a cell for determining susceptibility and resistance of a virus to the compound, comprises:

(a) obtaining nucleic acid encoding a viral envelope protein from a patient infected by the virus;

(b) co-transfecting into a first cell, the nucleic acid, and a viral expression vector which lacks a nucleic acid encoding an envelope protein, and which comprises an indicator nucleic acid which produces an detectable signal, such that the first cell produces viral particles comprising the envelope protein encoded by the nucleic acid obtained from the patient;

(c) contacting the viral particles with a second cell in the presence of the compound, where the second cell expresses a cell surface receptor to which the virus binds;

(d) measuring the amount of signal produced by the second cell in order to determine the infectivity of the viral particles; and

(e) comparing the amount of signal measured with the amount of signal produced in the absence of the compound, where a reduced amount of signal measured in the presence of the compound indicates that the compound inhibits entry of the virus into the second cell.

INDEPENDENT CLAIMS are also included for:

(1) making a composition by admixing the compound identified by the above method with a carrier;

(2) identifying a cell surface receptor which is bound by a virus upon infection of a cell by the virus, by obtaining viral particles which comprise a viral nucleic acid and an indicator nucleic acid which produces a detectable signal, contacting a cell which expresses a cell surface receptor with the viral particles, and measuring the amount of detectable signal produced within the cell, where production of the signal indicates the cell surface receptor expressed by the cell is bound by the virus, thus identifying the cell surface receptor as being bound by the virus upon infection of the cell; and

(3) identifying a mutation in a virus that confers resistance to a compound that inhibits viral entry into a cell, which involves determining the nucleic acid sequence or the amino acid sequence of the virus prior to any treatment of the virus with the compound, obtaining a virus resistant to the compound, determining the nucleic acid sequence or the amino acid sequence of the resistant virus, and comparing the nucleic acid sequence or the amino acid sequences, respectively, so as to identify the mutation in the virus that confers resistance to the compound.

USE - The method is useful for identifying whether a compound inhibits entry of a virus into a cell, where the compound binds to the cell surface receptor or the viral envelope protein and inhibits membrane fusion. The compound is a ligand of the cell surface receptor and comprises a peptide, peptidomimetic, organic molecule or synthetic compound, especially an **antibody**. The method is useful for determining susceptibility of a virus to a compound which inhibits viral cell entry and also for determining resistance of a virus to a compound which inhibits viral entry into a cell (claimed).

The method is useful for testing for dose-dependent inhibition of viral entry in the presence of inhibitors and testing for dose-dependent inhibition of infection in the presence of well characterized **HIV**-1 neutralizing **antibodies**. The drug susceptibility and resistance tests are useful for screening for compounds to treat viral diseases. The viral entry assay is useful to characterize the mechanism of action of new virus entry inhibitor drug candidates, and the antiviral activity against a spectrum of viruses that may differ in susceptibility.

ADVANTAGE - The method provides a rapid, sensitive phenotypic assay to measure the susceptibility of a virus to inhibitors of viral entry.

Dwg.0/9

L21 ANSWER 21 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-750429 [81] WPIDS
DNC C2002-212612
TI New immunogenic **HIV** peptide having one or more epitopes immunoreactive with cytotoxic T lymphocytes, useful for diagnosing, treating and monitoring **HIV** infection in humans.
DC B04 D16
IN BOND, K; DEGROOT, A; MCNICHOLL, J M; PAU, C; SRIWANTHANA, B
PA (UYBR-N) UNIV BROWN RES FOUND; (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 99
PI WO 2002069691 A2 20020912 (200281)* EN 65
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
AU 2002252166 A1 20020919 (200433)
ADT WO 2002069691 A2 WO 2002-US6314 20020301; AU 2002252166 A1 AU 2002-252166 20020301
FDT AU 2002252166 A1 Based on WO 2002069691
PRAI US 2001-272565P 20010301
AB WO 200269691 A UPAB: 20021216
NOVELTY - A new immunogenic **HIV** peptide (I) comprising 1 or more epitopes immunoreactive with cytotoxic T lymphocytes from an

**HIV** positive individual, is new. The peptide binds to **antibodies** that are immunoreactive with the assembled class I major histocompatibility complex and contains a sequence within the regions of the **HIV** genome according to the numbering in HXB2.

DETAILED DESCRIPTION - A new immunogenic **HIV** peptide (I) comprising 1 or more epitopes immunoreactive with cytotoxic T lymphocytes from an **HIV**-positive individual, is new. The peptide binds to **antibodies** that are immunoreactive with the assembled class I major histocompatibility complex and contains between 9 and eleven amino acid residues having a sequence within the regions of the **HIV** genome according to the numbering in HXB2. The residues of the peptide are within the regions of **HIV** genome according to the numbering in HXB2 selected from:

(a) pol 248-257, 272-281, 571-579;
(b) env gp120 6-15, 309-318, 340-348;
(c) env gp41 762-770;
(d) gag p17 83-91, 118-127;
(e) gag p15 376-384;
(f) pol 157-166, 894-903, 918-926;
(g) vif 84-93;
(h) tat 20-29;
(i) rev 107-115;
(j) env gp120 109-117, 244-252, 371-379, 433-442;
(k) env gp41 675-683;
(l) gag p17 19-28, 106-115;
(m) gag p24 221-229, 254-263, 256-264, 282-290, 352-361;
(n) gag p15 444-452;
(o) pol 158-166, 160-168, 169-177, 212-221, 229-238, 238-247, 249-258, 370-378, 370-379, 496-505, 612-620, 650-659, 751-760, 943-951, 991-999;
(p) vif 132-141, 159-168, 160-168, 160-169;
(q) **vpr** 4-12, 9-18;
(r) tat 80-89;
(s) rev 6-14;
(t) env gp120 162-171, 194-202, 194-203, 208-216, 208-217, 359-368, 413-421;
(u) env gp41 528-537, 721-729, 825-833; and
(v) nef 96-105, 109-118, 119-128, 147-156, 183-192, 188-197, 196-205.

INDEPENDENT CLAIMS are also included for:

(1) a method for monitoring the efficacy of a pharmaceutical anti-**HIV** drug comprising combining a sample from an **HIV**-positive patient to whom the drug has been administered with (I) and detecting the formation of a complex between the peptide and **antibodies** or T lymphocytes in the sample (the formation of a complex indicates efficacy);

(2) a prognosis method for determining AIDS resistance comprising combining a sample from an **HIV**-positive patient with (I) and detecting the formation of a complex between the peptide and **antibodies** or T lymphocytes in the sample (the detection of a complex indicates that the patient is resistant to progression of the disease to AIDS);

(3) a pharmaceutical composition comprising a carrier and (I); and

(4) a method of inducing an immune response in a human or animal comprising administering the pharmaceutical composition of (3).

ACTIVITY - Anti-HIV.

No biological data given.

MECHANISM OF ACTION - Gene therapy; Vaccine.

USE - The methods and compositions of the present invention are useful for diagnosing, treating and monitoring HIV infection in humans.

Dwg.0/1

L21 ANSWER 22 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-723330 [78] WPIDS
DNC C2002-204819
TI New recombinant modified vaccinia Ankara (MVA) virus expressing **HIV** env, gag and pol genes, useful for boosting or inducing CD8 T cell immune responses in primates, e.g. humans, particularly for preventing AIDS or other viral infections.

IN EARL, P; MOSS, B; WYATT, L
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 101
PI WO 2002072754 A2 20020919 (200278)* EN 112
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
EP 1372710 A2 20040102 (200409) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
AU 2002252199 A1 20020924 (200433)
ADT WO 2002072754 A2 WO 2002-US6713 20020301; EP 1372710 A2 EP 2002-721259 20020301, WO 2002-US6713 20020301; AU 2002252199 A1 AU 2002-252199 20020301
FDT EP 1372710 A2 Based on WO 2002072754; AU 2002252199 A1 Based on WO 2002072754
PRAI US 2001-274434P 20010308
AB WO 200272754 A UPAB: 20021204
NOVELTY - A composition, which comprises a recombinant modified vaccinia Ankara (MVA) virus expressing an **HIV** env, gag and pol gene, or its modified gene for the production of an **HIV** Env, Gag and Pol antigen by expression from the recombinant MVA virus, is new. The **HIV** env gene is modified to encode an **HIV** Env protein composed of gp120 and the membrane-spanning and ectodomain of gp41, but lacking part or all of the cytoplasmic domain of gp41.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) MVA/HIV48 comprising 74 bp (Psyn II promoter), 2214 bp (**HIV** env gene), 70 bp (PmH5 promoter) or 3479 bp (**HIV** gene) fully defined in the specification;

(2) the plasmid pLW-48 comprising 12225 bp fully defined in the specification;

(3) a plasmid transfer vector having the sequence of pLW-48, excluding the **HIV** env, gag and pol genes;

(4) a poxvirus comprising a promoter consisting of m7.5 promoter, Psyn II promoter, Psyn III promoter, Psyn IV promoter or Psyn V promoter, which comprise 93, 74, 74, 74 or 75 bp, respectively, fully defined in the specification;

(5) a method of boosting by administering the new composition or the MVA/HIV48 of (1);

(6) methods of inducing a CD+8 T cell immune response to an **HIV** Env, Gag or Pol antigen in a primate by:

(a) administering the new composition or the MVA/HIV48 of (1); or

(b) administering a priming composition comprising the nucleic acid encoding the antigen, and then administering the new composition or the MVA/HIV48 of (1); and

(7) a method of making the new composition.

ACTIVITY - Immunostimulant; Virucide.

MECHANISM OF ACTION - Vaccine.

The 89.6 chimera of simian and human immuno-deficiency virus (SHIC-89.6) was used for the construction of immunogens, and its highly pathogenic derivative (SHIV-89.6P) for challenge. Four groups of rhesus macaques were vaccinated by priming with DNA at 0 and 8 weeks, and boosting with recombinant MVA (rMVA) at 23 weeks. A control group included two mock immunized animals and two naive animals. The challenge was given at 7 months after the rMVA booster to test for the generation of long-term immunity. Results showed that 2 weeks after challenge, neutralizing **antibodies** for the 89.6 immunogen, but not the SIHV-89.6P challenge, were present in the high-dose DNA-primed groups. By 5 weeks, neutralizing **antibodies** to 89.6P had been generated, and those of 89.6 had started to decline. These results showed that multiprotein DNA/MVA vaccine raised a memory immune response capable of controlling a highly virulent mucosal

immune deficiency challenge.

USE - The composition or recombinant MVA virus is useful for boosting or inducing CD+8 T cell immune response in a primate, particularly in a human (claimed). The composition may be used for preventing AIDS or other viral infections.

Dwg.0/17

L21 ANSWER 23 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-722537 [78] WPIDS
CR 2000-037089 [03]
DNN N2002-569749 DNC C2002-204400
TI Limiting infection by an immunodeficiency virus, particularly **human immunodeficiency virus**, involves inhibiting an immunodeficiency virus protein which regulates apoptosis in T cells.
DC B04 D16 S03
IN CASELLA, C; FINKEL, T H
PA (NAJE-N) NAT JEWISH MEDICAL & RES CENT
CYC 1
PI US 2002091073 A1 20020711 (200278)* 13
ADT US 2002091073 A1 Provisional US 1995-9460P 19951229, Cont of US 1996-774269 19961227, Cont of US 1999-389944 19990903, US 2001-881573 20010613
PRAI US 1995-9460P 19951229; US 1996-774269 19961227; US 1999-389944 19990903; US 2001-881573 20010613
AB US2002091073 A UPAB: 20021204
NOVELTY - Limiting (M1) infection by an immunodeficiency virus comprises inhibiting an immunodeficiency virus protein which regulates apoptosis in cells.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) identifying (M2) a compound that regulates a cellular inhibitor of apoptosis in cells infected with an immunodeficiency virus involves:

(a) contacting a regulatory compound with cells infected with an immunodeficiency virus under conditions in which in the absence of the compound, apoptosis of the cells is inhibited, or with an immunodeficiency virus apoptosis inhibitor protein, and

(b) assessing the ability of the regulatory compound to regulate apoptosis in the cells where a difference in the rate of apoptosis between infected cells contacted with the compound compared to infected cells not in contact with the compound indicates that the compound regulates a cellular inhibitor of apoptosis in cells infected with the immunodeficiency virus, or assessing the ability of the compound to regulate the activity of the viral apoptosis inhibitor protein; and

(2) a compound identified by (M2).

ACTIVITY - Anti-**HIV**. No supporting data is given.

MECHANISM OF ACTION - Inhibitor of apoptosis in cells infected with immunodeficiency virus.

USE - (M1) is useful for limiting infection by an immunodeficiency virus such as **HIV**. (M2) is useful for identifying a compound that regulates a cellular inhibitor of apoptosis in T cells infected with **HIV** (claimed). Compounds identified by (M2) are useful as therapeutic reagents for treatment of immunodeficiency virus infections.

Dwg.0/2

L21 ANSWER 24 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-707002 [76] WPIDS
DNC C2002-200579
TI New chimeric gene, useful in anti-human immune deficiency virus vaccines, encodes T cell epitopes that are presented by a wide range of major histocompatibility alleles.
DC B04 D16
IN BLOMQUIST, D M V; CANO, C A D; PEREZ, E I; DUARTE CANO, C A; IGLESIAS PEREZ, E; VAZQUEZ BLOMQUIST, D M
PA (INGG-N) CENT ING GENETICA & BIOTECNOLOGIA; (CANO-I) DUARTE CANO C A;

CYC 101
PI WO 2002068654 A2 20020906 (200276)* ES 36
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
EP 1371730 A2 20031217 (200402) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
KR 2003079946 A 20031010 (200412)
BR 2002006823 A 20040225 (200416)
US 2004073008 A1 20040415 (200426)
AU 2002237195 A1 20020912 (200433)
ADT WO 2002068654 A2 WO 2002-CU1 20020222; EP 1371730 A2 EP 2002-703488 20020222, WO 2002-CU1 20020222; KR 2003079946 A KR 2003-709273 20030711; BR 2002006823 A BR 2002-6823 20020222, WO 2002-CU1 20020222; US 2004073008 A1 WO 2002-CU1 20020222, US 2003-469256 20030827; AU 2002237195 A1 AU 2002-237195 20020222
FDT EP 1371730 A2 Based on WO 2002068654; BR 2002006823 A Based on WO 2002068654; AU 2002237195 A1 Based on WO 2002068654
PRAI CU 2001-57 20010228
AB WO 200268654 A UPAB: 20021125
NOVELTY - Chimeric gene (CG) containing fragments from different **HIV**-1 (human immune deficiency virus) genes, is new.

DETAILED DESCRIPTION - Chimeric gene (CG) contains fragments from different **HIV**-1 (human immune deficiency virus) genes. The fragments encode regions rich in CTL (cytotoxic T lymphocyte) epitopes that are presented by a wide range of antigens of the MHC (major histocompatibility complex) type I, and may also contain selected epitopes for helper (Th) cells of **HIV**-1 and at least one B cell epitope of **HIV** recognized by a monoclonal **antibody**.

INDEPENDENT CLAIMS are also included for the following:

(1) chimeric protein (CP) having an amino acid (aa) sequence essentially the same as that encoded by gene cr3;

(2) recombinant virus (RV) containing CG as a heterologous gene;

(3) plasmids containing CG under control of a mammalian promoter; and

(4) vaccines containing RV or the plasmid of (3) and an excipient.

ACTIVITY - Anti-**HIV**; virucide.

B cells from **HIV**-infected patients were immortalized with Epstein-Barr virus, then infected with a vaccinia virus containing the preferred CG, cr3. They were then incubated with autologous peripheral blood cells (PBC) and the number of interferon gamma expressing cells specific for cr3 was determined by enzyme-linked immunospotting. The number was 400-900 per million PBC.

MECHANISM OF ACTION - Vaccine.

USE - Recombinant viruses and plasmids that contain CG are useful in vaccines for treatment or prevention of **HIV** infection.

ADVANTAGE - Unlike known minigenes, CG can present many epitopes, from several different proteins, simultaneously, so preparation of recombinant virus is simplified. No antibiotic resistance, or other irrelevant marker, genes are required, and epitopes from different viral strains can be combined in a single vaccine.

Dwg.0/8

L21 ANSWER 25 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-471505 [50] WPIDS
DNC C2002-134106
TI A nucleic acid construct with a sequence encoding a fusion protein comprising a destabilizing amino acid sequence attached to a heterologous amino acid, useful for gene therapy or vaccines against AIDS and AIDS-related diseases.

IN FELBER, B K; GRAGEROV, A; PAVLAKIS, G N
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 99
PI WO 2002036806 A2 20020510 (200250)* EN 73
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2002028722 A 20020515 (200258)
EP 1379535 A2 20040114 (200410) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI TR
ADT WO 2002036806 A2 WO 2001-US45624 20011101; AU 2002028722 A AU 2002-28722 20011101; EP 1379535 A2 EP 2001-989844 20011101, WO 2001-US45624 20011101
FDT AU 2002028722 A Based on WO 2002036806; EP 1379535 A2 Based on WO 2002036806
PRAI US 2000-245113P 20001101
AB WO 200236806 A UPAB: 20020807
NOVELTY - A nucleic acid construct (I) containing nucleotide sequences encoding a fusion protein, comprising a destabilizing amino acid sequence covalently attached to a heterologous amino acid (AA) sequence, in which the immunogenicity of the AA sequence is increased by the presence of the destabilizing amino acid sequence, is new.

DETAILED DESCRIPTION - A nucleic acid construct (I) contains nucleotide sequences encoding a fusion protein, and comprises a destabilizing amino acid sequence (which is present in the amino acid sequences selected from c-Myc aa2-120, Cyclin A aa13-91, Cyclin B 10-95, Cyclin B aa13-91, IkBa aa20-45, beta -Catenin aa19-44, c-Jun aa1-67, and c-Mos aa1-35) covalently attached to a heterologous amino acid (AA) sequence, in which the immunogenicity of the AA sequence is increased by the presence of the destabilizing amino acid sequence.

INDEPENDENT CLAIMS are also included for the following:

(1) a vector comprising (I);

(2) a host cell comprising (I);

(3) a composition comprising (I) and a pharmaceutical carrier;

(4) a vaccine composition for inducing immunity in a mammal against **HIV** infection comprising (I) and a pharmaceutical carrier;

(5) a viral particle comprising (I);

(6) a pharmaceutical composition comprising the viral particle;

(7) a composition comprising one or more vectors expressing different forms of an antigen covalently linked to destabilizing or secreting groups;

(8) a fusion polypeptide encoded by (I);

(9) a method of inducing **antibodies** in a mammal, stimulating the immune response against an amino acid sequence of interest, or inducing cytotoxic and/or helper-inducer T lymphocytes, by administering to a mammal a composition of (3);

(10) a method for inducing immunity against **HIV** infection in a mammal by administering the vaccine of (4);

(11) a method of stimulating the immune response against an amino acid sequence of interest by administering the composition of (6);

(12) a composition comprising one or more vectors expressing different forms of an antigen covalently linked to destabilizing or secreting moieties;

(13) a method for inducing **antibodies** or cytotoxic and/or helper-inducer T lymphocytes in a mammal by administering a composition of (12);

(14) a composition containing the vectors comprising nucleic acids which encode wt gag, MCP3gag, and B-CATEgag; and

(15) a composition comprising the vectors wt env, MCP3env, and B-CATEenv.

ACTIVITY - Anti-**HIV**; Immunostimulant.

MECHANISM OF ACTION - Vaccine; Gene therapy.

Three groups of 4 naive macaques were injected with SIV DNA vaccine:

(1) SIVgagDX, pCMVkan/R-R-SIVgp160CTE, SIVMCP3p39, and pCMVkan/MCP3/SIVgp160CTE;

(2) SIVgagDX, pCMVkan/R-R-SIVgp160CTE, SIVCATEp39, and pCMVkan/CATE/SIVgp160CTE; and

(3) SIVgagDX, pCMVkan/R-R-SIVgp160CTE, SIVMCP3p39, pCMVkan/MCP3/SIVgp160CTE, SIVCATEp39, and pCMVkan/CATE/SIVgp160CTE.

Four animals (group 4) were immunized first with SIVCATEp39, and pCMVkan/CATE/SIVgp160CTE, and at weeks 12 and 24 with SIVMCP3p39 and pCMVkan/MCP3/SIVgp160CTE. Two animals (group 5) received DNAs expressing unmodified wild-type antigens for gag and env (SIVgagDX and pCMVkan/R-R-SIVgp160CTE). The total amount of DNA injected each time per animal was kept constant at 3 mg for gag and 3 mg for env. Animals were injected at different sites with the different DNAs. Injections were intramuscular with the DNA delivered in phospate buffered saline at 1 mg/ml. The sites were anatomically separate for the different DNAs. Four animals in group 4 were immunized first with DNAs 5 and 6, and subsequently at weeks 12 and 24 with DNAs 3 and 4. Two animals in group 5 received the DNAs expressing unmodified, wild-type antigens for gag and env (1 and 2). The animals in groups 4 and 5 had been previously exposed to **HIV** DNA, but they were nave for SIV antigens, which was verified by immunological assays. Animals in groups 4 and 5 showed early responses to SIV DNA injection, indicating an anamnestic response to SIV antigens. At sequential times during vaccination, blood samples were obtained and analyzed for the presence of **antibodies**, lymphoproliferative responses and cytotoxic T cells. Results showed that administration of MCP3gag vector is associated with strong **antibody** response, because 8 out of 8 or 100% of animals receiving MCP3gag (in groups 1 and 3) developed high gag **antibodies**. In contrast, 3 out of 6 or 50% of animals not receiving MCP3gag (in groups 2 and 5) developed **antibodies**. The specific cytotoxic T cell responses against gag and env were evaluated by measuring the number of CD8 cells that produce intracellular IFNgamma or TNFalpha in the presence of gag or env synthetic peptide pools. It was concluded that the animals receiving all three forms of antigens showed increased **antibody** response without diminishing cellular immune response. The cellular immune response also showed increased cellular immune response and the results showed statistical significant differences. The data indicate the development of a more balanced immune response than previously anticipated by DNA vaccination in macaques, by the combination of different antigen forms.

USE - The constructs are useful for in vivo or in vitro gene therapy, in the development of vaccines, and in the production of diagnostic reagents, vaccines and therapies for diseases such as acquired immune deficiency syndrome (AIDS) and AIDS-related diseases. The nucleic acids, vectors, vector systems, host cells and compositions can be used to produce mRNA, polypeptides, and/or infectious viral particles; to induce **antibodies**, cytotoxic and/or helper T lymphocytes; in immunotherapy and immunoprophylaxis, e.g. as vaccine, or in genetic therapy after expression in mammals.

ADVANTAGE - A combination of DNA vaccines containing different antigen forms and administering at different sites, increases the immune response.

Dwg.0/10

L21 ANSWER 26 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-426956 [45] WPIDS
CR 2003-335345 [32]; 2003-335346 [32]; 2003-335347 [32]; 2003-335348 [32]; 2003-335349 [32]; 2003-335350 [32]
DNC C2002-121140
TI Analyzing phenotype of human immune deficiency virus, useful for optimizing therapy, by cloning segment into viral particle and transfecting cell containing inducible marker gene.
DC B04 D16
IN CLAVEL, F; DAM, E; MAMMANO, F; OBRY, V; RACE, E; TROUPLIN, V
PA (BIOA-N) BIOALLIANCE PHARMA; (INRM) INSERM INST NAT SANTE & RECH MEDICALE;

(MAMM-I) MAMMANO F; (OBRY-I) OBRY V; (RACE-I) RACE E; (TROU-I) TROUPLIN V

CYC 99

PI WO 2002038792 A2 20020516 (200245)* FR 98
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
FR 2816634 A1 20020517 (200245)
FR 2816635 A1 20020517 (200245)
AU 2002023052 A 20020521 (200260)
US 2002123036 A1 20020905 (200260)
US 2003207294 A1 20031106 (200374)
EP 1364071 A2 20031126 (200380) FR
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
US 2004053219 A1 20040318 (200421)
US 2004101828 A1 20040527 (200435)

ADT WO 2002038792 A2 WO 2001-FR3512 20011109; FR 2816634 A1 FR 2000-14495 20001110; FR 2816635 A1 FR 2001-3970 20010323; AU 2002023052 A AU 2002-23052 20011109; US 2002123036 A1 US 2001-817135 20010327; US 2003207294 A1 Div ex US 2001-817135 20010327, US 2002-263655 20021004; EP 1364071 A2 EP 2001-993700 20011109, WO 2001-FR3512 20011109; US 2004053219 A1 Cont of US 2001-817135 20010327, US 2003-436458 20030513; US 2004101828 A1 Cont of WO 2001-FR3512 20011109, US 2003-435659 20030512

FDT AU 2002023052 A Based on WO 2002038792; EP 1364071 A2 Based on WO 2002038792

PRAI US 2001-817135 20010327; FR 2000-14495 20001110; FR 2001-3970 20010323

AB WO 200238792 A UPAB: 20040603

NOVELTY - Analyzing phenotype of **HIV** (human immune deficiency virus), resulting from one or more mutations in the viral genome that influence infection, in a patient sample, is new.

DETAILED DESCRIPTION - Analyzing phenotype of **HIV** (human immune deficiency virus), resulting from one or more mutations in the viral genome that influence infection, in a patient sample, is new. Nucleic acids are extracted from the sample, segments of them amplified by PCR (polymerase chain reaction) using pairs of primers that flank a genomic sequence susceptible to mutation, and a first host cell (HC1) transfected with:

(a) the amplicon;

(b) a vector containing parts of the **HIV** genome required for replication, except for the amplified segment and optionally also the env gene; and

(c) if the vector of (b) lacks the env gene, also a second vector containing this gene.

Homologous recombination occurs to produce a chimeric virus and HC1 are cultured to produce viral particles (VP) during a single cycle of replication. VP are used to infect at least one second host cell (HC2) that contains a marker gene (MG) that is activated only after viral infection, then the expressed marker detected and/or quantified to detect at least one characteristic of the original **HIV**.

An INDEPENDENT CLAIM is also included for a kit for performing the new process.

USE - The method is used to characterize **HIV** for optimization of treatment.

ADVANTAGE - The method allows rapid testing (7 days, making it suitable for routine use) of phenotypic characteristics associated with infectivity, replicative capacity and virulence, susceptibility/resistance to antiretroviral agents or natural **antibodies**, and tropism for particular co-receptors. The method requires only a single round of replication, reducing the risk that mutations will be lost.

Dwg.0/6

L21 ANSWER 27 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-329780 [36] WPIDS
DNC C2002-095344
TI Stable transduction of cells useful in research or for treatment or prevention diseased conditions in living subjects, by contacting cells with lentiviral vector and cell surface binding molecule.
DC B04 D16
IN CHANG, Y; DAVIS, B; DROPULIC, B; HAN, W; HUMEAU, L; LESHER, M; LU, X; SLEPUSHKIN, V; STEPUSHKIN, V
PA (VIRX-N) VIRXSYS; (VIRX-N) VIRXSYS CORP; (CHAN-I) CHANG Y; (DAVI-I) DAVIS B; (DROP-I) DROPULIC B; (HANW-I) HAN W; (HUME-I) HUMEAU L; (LESH-I) LESHER M; (LUXX-I) LU X; (SLEP-I) SLEPUSHKIN V
CYC 97
PI WO 2002018609 A2 20020307 (200236)* EN 41
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001086947 A 20020313 (200249)
EP 1315823 A2 20030604 (200337) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
NO 2003000901 A 20030428 (200340)
US 6627442 B1 20030930 (200367)
CZ 2003000569 A3 20031112 (200379)
KR 2003074592 A 20030919 (200409)
BR 2001013619 A 20040406 (200425)
US 2004062756 A1 20040401 (200425)
ADT WO 2002018609 A2 WO 2001-US27091 20010829; AU 2001086947 A AU 2001-86947 20010829; EP 1315823 A2 EP 2001-966434 20010829, WO 2001-US27091 20010829; NO 2003000901 A WO 2001-US27091 20010829, NO 2003-901 20030226; US 6627442 B1 US 2000-653088 20000831; CZ 2003000569 A3 WO 2001-US27091 20010829, CZ 2003-569 20010829; KR 2003074592 A KR 2003-702964 20030227; BR 2001013619 A BR 2001-13619 20010829, WO 2001-US27091 20010829; US 2004062756 A1 Cont of US 2000-653088 20000831, US 2003-664331 20030916
FDT AU 2001086947 A Based on WO 2002018609; EP 1315823 A2 Based on WO 2002018609; CZ 2003000569 A3 Based on WO 2002018609; BR 2001013619 A Based on WO 2002018609; US 2004062756 A1 Cont of US 6627442
PRAI US 2000-653088 20000831; US 2003-664331 20030916
AB WO 200218609 A UPAB: 20020610
NOVELTY - Stable transduction (M1) of cell by contacting the cells with a lentiviral vector (LV) and at least one cell surface binding molecule (CSBM), where greater than 75% of the cells are stably transduced after about 14 days, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for introducing genetic material into a living subject comprising introduction of a cell transduced by M1.

USE - M1 is useful for stable transduction of cell (claimed), where the transduced cell is useful for producing a desired gene products and proteins by expression of a nucleic acid present in the vector, or for therapy of living subjects afflicted, or at risk of being afflicted with the disease and transduced cells may further be utilized in research or for treatment or prevention diseased conditions in living subjects.

ADVANTAGE - M1 stably transduces cells preferably at greater than 75% after about 14 days, and further eliminates the purification of the cell to be transduced.

Dwg.0/9

L21 ANSWER 28 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2002-114343 [15] WPIDS
DNC C2002-035092
TI Simian immunodeficiency virus-derived vector for nucleic acid transfer,

comprises a transfer vector having a nucleotide of interest, a packaging vector without accessory proteins, and an env vector encoding an envelope protein.

DC B04 C06 D16

IN PLANELLES, V

PA (UYRP) UNIV ROCHESTER

CYC 86

PI WO 2001092506 A1 20011206 (200215)* EN 39
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 2001065190 A 20011211 (200225)
US 2003134817 A1 20030717 (200348)

ADT WO 2001092506 A1 WO 2001-US17458 20010530; AU 2001065190 A AU 2001-65190 20010530; US 2003134817 A1 Provisional US 2000-207916P 20000530, Provisional US 2001-287433P 20010430, Cont of WO 2001-US17458 20010530, US 2002-304988 20021126

FDT AU 2001065190 A Based on WO 2001092506

PRAI US 2001-287433P 20010430; US 2000-207916P 20000530; US 2002-304988 20021126

AB WO 200192506 A UPAB: 20020306

NOVELTY - A Simian immunodeficiency virus (SIV)-derived vector system (I) for transferring a nucleic acid encoding a target molecule into a host cell, comprising a transfer vector containing a nucleic acid segment of interest, a packaging vector which is deleted for one of the accessory proteins, and an env vector containing an envelope protein which is not an SIV envelope protein, is new.

DETAILED DESCRIPTION - A Simian immunodeficiency virus (SIV)-derived vector system (I) comprises:

(a) a transfer vector (TV) containing a nucleic acid sequence encoding a target molecule, where the nucleic acid sequence is operably linked to a promoter and a SIV packaging sequence including the portion of the SIV long terminal repeat (LTR) sequences necessary to package the SIV RNA into the SIV virion;

(b) a packaging vector (PV) derived from an SIV strain and which has at least one accessory gene deleted, which further contains a SIV gag gene encoding a gag protein, where the gag gene is operably linked to a promoter and a polyadenylation sequence;

(c) an env vector (EV) containing an env gene encoding a functional envelope protein from a virus other than a lentivirus, where the env gene is operably linked to a promoter and a polyadenylation sequence; and

(d) an SIV pol gene encoding a pol protein on one of the first two vectors or on at least a third vector, where the lentiviral pol gene is operably linked to a promoter and a polyadenylation sequence, where only TV contains the SIV packaging segment to effectively package lentiviral RNA, and the SIV protein and the envelope protein when expressed in combination form a SIV virion containing an envelope protein around a SIV plasmid.

An INDEPENDENT CLAIM is also included for a host cell transfected by (I).

ACTIVITY - None given.

MECHANISM OF ACTION - Gene therapy. No biological data is given.

USE - (I) is useful for transferring a nucleic acid encoding a target molecule into a host cell (claimed).

ADVANTAGE - The vectors are able to infect a host cell with high efficiency and have a reduced threat of being a human pathogen. The vector system can be used to package a wide range of desired nucleotide segments, into an empty lentiviral particle because of the relatively large genomes of lentiviruses.

Dwg.0/3

L21 ANSWER 29 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text

DNC C2001-111101
TI New vectors comprising or expressing at least one immortalization molecule, useful for stably integrating a transgene in the genome of non-dividing (n-d) or slowly dividing (s-d) cells or for expanding n-d or s-d cells.
DC B04 D16
IN OCCHIDORO, T; SALMON, P; TRONO, D; OCCHIODORO, T
PA (UYGE-N) UNIV GENEVE
CYC 95
PI EP 1103615 A1 20010530 (200138)* EN 29
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
WO 2001038548 A2 20010531 (200138) EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001026679 A 20010604 (200153)
EP 1244798 A2 20021002 (200265) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
JP 2003514565 W 20030422 (200336) 67
ADT EP 1103615 A1 EP 1999-123498 19991125; WO 2001038548 A2 WO 2000-EP11723 20001124; AU 2001026679 A AU 2001-26679 20001124; EP 1244798 A2 EP 2000-989880 20001124, WO 2000-EP11723 20001124; JP 2003514565 W WO 2000-EP11723 20001124, JP 2001-539890 20001124
FDT AU 2001026679 A Based on WO 2001038548; EP 1244798 A2 Based on WO 2001038548; JP 2003514565 W Based on WO 2001038548
PRAI EP 1999-123498 19991125
AB EP 1103615 A UPAB: 20010711
NOVELTY - A vector (I), which is capable of stably integrating a transgene in the genome of a n-d cell or of a s-d cell, is new. The vector comprises or expresses at least one immortalization molecule.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) producing (I);

(2) the immortalization (M1) of n-d or s-d cells comprising introducing into the n-d or slow-dividing at least one of the vectors above, and expressing the immortalization molecules encoded by the vector(s) in the n-d or s-d cells;

(3) a stable packaging cell line producing a vector;

(4) immortalized cells obtainable by (M2);

(5) cells originally n-d or s-d characterized in that they are immortalized, or cells characterized in that they are immortalized and do not lose irreversibly their original phenotype of interest;

(6) bioartificial pancreas or skin comprising the cells, or myotubules formed from the fusion of cells; and

(7) producing (M2) the protein of interest characterized in that immortalized cells are cultured in vitro or ex vivo, under conditions permitting the expression of the protein of interest in the cell, and optionally recovering the protein.

USE - The vectors are useful for expanding non-dividing or slowly dividing cells in vitro. The cells are useful for producing monoclonal **antibodies** or a protein of interest in vitro, in pharmacological study, for treating burn or ulcers, or for reconstituting muscles. The cells, particularly dendritic cells, are also useful for designing anti-tumoral or anti-infectious substances (all claimed).

Dwg.0/5

L21 ANSWER 30 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2001-032041 [04] WPIDS
DNC C2001-009865

TI Inhibiting or preventing infectious agent transmission in mammalian transplant recipients, by introducing recombinant DNA comprising DNA encoding extracellular proteins of the agent into donor cells, such as swine cells.

DC B04 D16

IN FEDERSPIEL, M J

PA (MAYO-N) MAYO MEDICAL VENTURES

CYC 93

PI WO 2000071726 A1 20001130 (200104)* EN 143
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000052865 A 20001212 (200115)

ADT WO 2000071726 A1 WO 2000-US14296 20000524; AU 2000052865 A AU 2000-52865 20000524

FDT AU 2000052865 A Based on WO 2000071726

PRAI US 1999-135631P 19990524

AB WO 200071726 A UPAB: 20010118

NOVELTY - Inhibiting or preventing infectious agent transmission in a mammalian transplant recipient, comprising introducing to donor swine cells, human blood cells, or organs, recombinant DNA comprising a promoter operably linked to DNA encoding at least part of a polypeptide present in the extracellular form of an infectious agent, and introducing the transformed cells, or organ, to the recipient, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated and purified nucleic acid, comprising a segment comprising at least a portion of a pig endogenous retrovirus, the segment hybridizes under hybridizing conditions to a 1980, 7362, 4402, 6076, 4918, 7873 or 6076 nucleotide sequence (S1), all fully defined in the specification, and encoding pig endogenous retroviruses (PERV);

(2) an isolated polypeptide encoded by a nucleic acid comprising a segment of (S1);

(3) detecting human tropic PERVs, comprising:

(a) contacting a mammalian sample with a probe comprising at least a portion of (S1); and

(b) detecting or determining the presence of complexes;

(4) a host cell whose genome is augmented with a recombinant DNA comprising a promoter operably linked to a DNA segment encoding a fusion protein comprising at least part of a PERV and a degradative enzyme;

(5) a fusion protein comprising a capsid or envelope protein of a PERV and a degradative enzyme;

(6) an isolated and purified DNA encoding the fusion protein of (5);

(7) a recombinant virus comprising the nucleic acid of (6); and

(8) an **antibody** specific for PERVs.

ACTIVITY - Antiviral.

No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - For inhibiting or preventing agent transmission in a mammalian transplant recipient. The transplant is mammalian cells, preferably human or swine cells, especially human blood cells, or an organ. The agent is a virus, particularly a lentivirus, retrovirus, PERV, hepatitis virus, herpesvirus, Epstein-Barr virus, cytomegalovirus or **human immunodeficiency virus** (**HIV**). PERV DNA sequences can be used probes to detect human tropic PERVs. (All claimed).

ADVANTAGE - The method prevents or inhibits transmission of infectious agents such as PERVs from the donor to the recipient, after cell, tissue or organ transplant, reducing the risks involved in transplantation, particularly xenotransplantation. The use of degradative enzymes rather than overexpression of a mutant protein which interferes with the viral life cycle, reduces the risk of viral resistance.

Dwg.0/18

L21 ANSWER 31 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2000-565367 [52] WPIDS
DNC C2000-168414
TI New synthetic peptides from the **Vpr** protein of human immune deficiency virus, useful e.g. for therapy and diagnosis, have good solubility in water.
DC B04 D16
IN HENKLEIN, P; SCHUBERT, U; WRAY, V
PA (GLAD-N) GLADSTONE INST J DAVID; (HENK-I) HENKLEIN P; (SCHU-I) SCHUBERT U; (WRAY-I) WRAY V
CYC 22
PI WO 2000049038 A2 20000824 (200052)* GE 34
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: JP US
DE 19908752 A1 20000831 (200052)
DE 19908766 A1 20000831 (200052)
DE 19908766 C2 20010215 (200110)
EP 1155035 A2 20011121 (200176) GE
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
JP 2002540768 W 20021203 (200309) 43
CA 2356390 A1 20030217 (200329)# EN
ADT WO 2000049038 A2 WO 2000-DE525 20000219; DE 19908752 A1 DE 1999-1008752 19990219; DE 19908766 A1 DE 1999-1008766 19990219; DE 19908766 C2 DE 1999-1008766 19990219; EP 1155035 A2 EP 2000-918674 20000219, WO 2000-DE525 20000219; JP 2002540768 W JP 2000-599775 20000219, WO 2000-DE525 20000219; CA 2356390 A1 CA 2001-2356390 20010817
FDT EP 1155035 A2 Based on WO 2000049038; JP 2002540768 W Based on WO 2000049038
PRAI DE 1999-19908766 19990219; DE 1999-19908752 19990219; CA 2001-2356390 20010817
AB WO 200049038 A UPAB: 20001018
NOVELTY - Synthetic peptides (I) of the regulatory **viral protein R** (**Vpr**) of **human immunodeficiency virus**-1 (**HIV**-1), are new.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for preparation of (I) by solid-phase synthesis.
ACTIVITY - Antiviral. No biological data is given.
MECHANISM OF ACTION - Gene therapy.
USE - (I) is used for therapeutic and/or diagnostic purposes, especially in biological assays, for development of serological tests or enzyme-linked immunosorbent assays (ELISA) (e.g. for detecting or quantifying **Vpr** in blood), to raise specific **antibodies** and antisera (especially those reactive with specific epitopes), and as antiviral agents. (I) can also be used in screening for potential **Vpr** antagonists (i.e. compounds that modulate interaction of **Vpr** with cellular factors, transcription-activating properties of **Vpr**, transport of **Vpr** and its incorporation into viral particles, **Vpr**-induced cell cycle arrest, and cytotoxic and ion-channel activities of **Vpr**). (I) is used to establish cell or animal models for studying pathogenicity of **Vpr**, for structural analysis of **Vpr** and its domains, for in vitro assembly of new vectors for gene therapy, in vitro or in vivo, for complementing the function of **Vpr**-defect mutants in cell cultures, and to reduce flexibility of **Vpr** induced by the N-terminal domain. All claimed.
ADVANTAGE - Synthetic (I), are soluble in water and can be formulated as highly concentrated solutions (mmolar) without protein aggregation, so are well suited to analysis by nuclear magnetic resonance, X-ray or circular dichroism techniques. (I) adopt a folded structure, have biological activity comparable to that of viral **Vpr**, and can be produced, at high purity, on the milligram scale.
Dwg.0/10

L21 ANSWER 32 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2000-365651 [31] WPIDS
DNC C2000-110513
TI Novel genomic nucleic acids of non-subtype B **human immunodeficiency**

virus type 1 useful for detecting and treating AIDS comprises a specific nucleotide sequence.

DC B04 D16

IN GAO, F; HAHN, B H; SHAW, G M

PA (UABR-N) UAB RES FOUND

CYC 90

PI WO 2000026416 A1 20000511 (200031)* EN 141

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000012241 A 20000522 (200040)

US 6492110 B1 20021210 (200301)

US 2003148266 A1 20030807 (200358)

ADT WO 2000026416 A1 WO 1999-US24837 19991025; AU 2000012241 A AU 2000-12241 19991025; US 6492110 B1 US 1998-184418 19981102; US 2003148266 A1 Div ex US 1998-184418 19981102, US 2002-290579 20021108

FDT AU 2000012241 A Based on WO 2000026416; US 2003148266 A1 Div ex US 6492110

PRAI US 1998-184418 19981102; US 2002-290579 20021108

AB WO 200026416 A UPAB: 20000630

NOVELTY - A nucleic acid sequence (I) comprising a sequence of 8959 base pairs (as given in the specification) of the genome of a non-subtype B **human immunodeficiency virus** type 1 (**HIV**-1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid (Ia) comprising a sequence of at least 12 contiguous bases or LTR derived from (I);

(2) a nucleic acid sequence (Ib) encoding polypeptide Gag, Pol, Vif, **Vpr**, Env, Tat, Rev, Nef or Vpu which is encoded by the genome of virus 92RW009.6, 92NG003.1, 92NG083.2, 93BR020.1, 93BR029.4, 90CF056.1, 94CY032.3, 94CY017.41, 96ZM651.8, 96ZM751.3 or 94IN476.104;

(3) a nucleic acid (Ic) comprising a sequence complementary to (I)-(Ib);

(4) a vector (II) comprising (I)-(Ic);

(5) a cell (III) comprising (I)-(Ic) or (II);

(6) a polypeptide (PP) encoded by (I);

(7) a nucleic acid probe (Id) comprising a sequence of at least 19 contiguous nucleotides of a 8959 base pair sequence;

(8) production (P) of PP;

(9) inducing serum **antibodies** (A) that bind PP by administering PP or (I) into mammals;

(10) (A) against **HIV**-1 produced by the above method;

(11) a kit for detecting the presence of non-subtype B **HIV**-1 virus in a sample comprising (A) or (I)-(Id);

(12) a composition (C) comprising (I)-(Id), (PP) or (A); and

(13) analyzing a first nucleotide or a first amino acid sequence by comparing with a 8959 base pair sequence or a 491 amino acid sequence, respectively.

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - Vaccine. No supporting data given.

USE - PP is useful for producing (A) against non-subtype B **HIV**-1 (claimed). (A) is useful for detecting **HIV**-1 virus in the sample by forming antigen-**antibody** complexes. PP and (I) are also useful for detecting the presence of non-subtype B **HIV**-1 in biological samples. (Id) is also useful for detecting the presence of non-subtype B **HIV**-1 virus in the biological sample by detecting the complex between nucleic acid and the sample and (Id) when one or more (Id) is contacted (claimed). PP is also useful as immunogens and as diagnostic agents.

ADVANTAGE - The study of genomic clones of **HIV**-1 provides a significant impact on immune protections and subtype specific reagents which are critically needed for phylogenetic, immunological and biochemical studies.

Dwg.0/22

L21 ANSWER 33 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2000-037081 [03] WPIDS
CR 2000-125951 [11]; 2001-060089 [02]
DNC C2000-009448
TI Composition containing an antigen and altered major histocompatibility Class II determinant, used to immunize against autoimmune diseases, e.g. acquired immune deficiency syndrome.
DC B04 D16
IN ABASTADO, J; KOURILSKY, P; MOTTEZ, E
PA (INRM) INSERM INST NAT SANTE & RECH MEDICALE; (INSP) INST PASTEUR
CYC 1
PI US 5976551 A 19991102 (200003)* 96
ADT US 5976551 A Cont of US 1991-792473 19911115, Div ex US 1991-801818 19911205, US 1995-484905 19950607
PRAI US 1991-792473 19911115; US 1991-801818 19911205; US 1995-484905 19950607
AB US 5976551 A UPAB: 20010202
NOVELTY - A composition capable of eliciting anti-major histocompatibility (MHC) **antibodies** comprises an antigen associated with an altered MHC Class II determinant (I).

DETAILED DESCRIPTION - A composition that can elicit anti-MHC **antibodies** comprises an antigen associated with an altered MHC Class II determinant (I) comprising alpha 1, alpha 2, beta 1 and beta 2 polypeptide domains encoded by a mammalian MHC Class II locus covalently linked to form a polypeptide (I) containing beta 2, alpha 2, alpha 1 and beta 1 domains in sequence.

The resulting Antigen-MHC complex is recognizable by the T cell receptor.

ACTIVITY - Cytostatic; anti-inflammatory; neuroprotective; dermatological; immunosuppressive; antithyroid.

No relevant biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The compositions are used for immunization against, or treatment of, a wide range of autoimmune diseases, e.g. acquired immune deficiency syndrome (AIDS), lupus erythematosus, multiple sclerosis, thyroiditis, toxic shock, tumor and snakebite, depending on the nature of antigen. (I) is also used to analyze functional interactions between the various domains and for targeting lymphocyte receptors.

**Antibodies** against (I) are produced by usual methods of immunization or cell fusion, and may be humanized by standard methods. These **antibodies** are useful for diagnosis (detection or purification of MHC gene products), therapy (neutralizing MHC on cell surfaces) and in the study of MHC and cellular processes.

ADVANTAGE - The compositions specifically redirect the target-recognition potential of immune system cells to antigen, and may contain more than one (I)-antigen product for simultaneous or sequential targeting of different receptors. (I) are easier to manipulate than native MHC molecules and can be refolded during and after denaturing treatments. Immune system cells recognize (I) and generate an effective response typical of their lineage (e.g. cytotoxicity, **antibody** production, cytokine secretion etc.).

Dwg.0/6

L21 ANSWER 34 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1999-407452 [35] WPIDS
DNC C1999-120602
TI Carboxymethylated viral regulatory proteins or interferon, for treatment and prevention of retroviral infection, specifically by human immune deficiency virus.
DC A96 B04 C06 D16
IN CARCAGNO, M; RAPPAPORT, J; ZAGURY, J F
PA (BIOV-N) BIOVACS INC; (CARC-I) CARCAGNO M; (RAPP-I) RAPPAPORT J; (ZAGU-I) ZAGURY J; (NEOV-N) NEOVACS
CYC 85

WO 9933346 A1 19990708 (199939) EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
WO 9933872 A1 19990708 (199939) FR
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9915684 A 19990719 (199951)
AU 9919397 A 19990719 (199951)
EP 1041888 A1 20001011 (200052) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
EP 1042363 A1 20001011 (200052) FR
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
BR 9814473 A 20011023 (200172)
JP 2001527029 W 20011225 (200204) 44
US 6420141 B1 20020716 (200248)
MX 2000006263 A1 20030701 (200420)

ADT FR 2773156 A1 FR 1997-16589 19971226; WO 9933346 A1 WO 1998-US27263 19981222; WO 9933872 A1 WO 1998-FR2727 19981214; AU 9915684 A AU 1999-15684 19981214; AU 9919397 A AU 1999-19397 19981222; EP 1041888 A1 EP 1998-964219 19981222, WO 1998-US27263 19981222; EP 1042363 A1 EP 1998-959984 19981214, WO 1998-FR2727 19981214; BR 9814473 A BR 1998-14473 19981222, WO 1998-US27263 19981222; JP 2001527029 W WO 1998-US27263 19981222, JP 2000-526120 19981222; US 6420141 B1 WO 1998-FR2727 19981214, US 2001-582519 20010315; MX 2000006263 A1 WO 1998-US27263 19981222, MX 2000-6263 20000623

FDT AU 9915684 A Based on WO 9933872; AU 9919397 A Based on WO 9933346; EP 1041888 A1 Based on WO 9933346; EP 1042363 A1 Based on WO 9933872; BR 9814473 A Based on WO 9933346; JP 2001527029 W Based on WO 9933346; US 6420141 B1 Based on WO 9933872; MX 2000006263 A1 Based on WO 9933346

PRAI FR 1997-16589 19971226

AB FR 2773156 A UPAB: 19990902

NOVELTY - Carboxymethylated protein (I), or its fragments, is derived from a viral regulatory protein (Ia) or alpha -interferon (Ib).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) method for producing (I) by carboxymethylation of the parent protein, or fragment;

(2) pharmaceutical composition containing (I) and an excipient; and

(3) vaccine containing at least one (I).

ACTIVITY - Antiviral; anti-immunosuppressive.

MECHANISM OF ACTION - (I) induce a specific **antibody** response against the parent protein.

USE - (I) are used as 'toxoids' for treatment or prevention, as vaccines, of the effects associated with overexpression of (Ib) or retroviral (Ia), in particular for preventing the immunosuppression, characteristic of acquired immune deficiency syndrome, caused by human immune deficiency virus (**HIV**). Alternatively, **antibodies**, or their fragments, raised against (I) can be used therapeutically. A hemophiliac seropositive for **HIV**-1 was immunized with carboxymethylated Tat protein (0.1 mg active ingredient). Initial CD4 levels were 282 cells/cubic mm and viremia was 3.34, but at the end of the six month trial these figures were 319 cells/cubic mm and 0.91. The level of **HIV**-1 antigen was unchanged. The basal and peak levels of anti-TAT **antibodies**, expressed as optical density units, were 0.446 and 1.612, respectively.

ADVANTAGE - Carboxymethylated proteins are biologically inactive (especially they do not have the immunosuppressive effects of their parent

proteins, but remain immunogenic to induce neutralizing/blocking **antibodies** against the parent. Carboxymethylated Tat protein (20 mu g) was used to immunize mice (day 0), with 5 mu g boosters given on days 21 and 35. Analysis by enzyme-linked immunosorbent assay for anti-TAT **antibodies** indicated the same response as with native Tat and that the induced **antibodies** were neutralizing.
Dwg.0/1

L21 ANSWER 35 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1999-395185 [33] WPIDS
DNC C1999-116199
TI A modified **human immunodeficiency virus** genome.
DC B04 D16
IN CAO, S X; ROVINSKI, B; YAO, F
PA (CONN-N) CONNAUGHT LAB LTD; (AVET) AVENTIS PASTEUR LTD
CYC 83
PI WO 9931250 A2 19990624 (199933)* EN 36
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9916577 A 19990705 (199948)
EP 1038001 A2 20000927 (200048) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
US 6121021 A 20000919 (200048)
MX 2000005700 A1 20010201 (200168)
AU 749174 B 20020620 (200252)
BR 9813626 A 20020723 (200257)
US 6572863 B1 20030603 (200339)
JP 2004509601 W 20040402 (200424) 59
ADT WO 9931250 A2 WO 1998-CA1164 19981214; AU 9916577 A AU 1999-16577 19981214; EP 1038001 A2 EP 1998-960976 19981214, WO 1998-CA1164 19981214; US 6121021 A US 1997-991773 19971216; MX 2000005700 A1 MX 2000-5700 20000609; AU 749174 B AU 1999-16577 19981214; BR 9813626 A BR 1998-13626 19981214, WO 1998-CA1164 19981214; US 6572863 B1 Cont of US 1997-991773 19971216, WO 1998-CA1164 19981214, US 2000-555834 20000719; JP 2004509601 W WO 1998-CA1164 19981214, JP 2000-539149 19981214
FDT AU 9916577 A Based on WO 9931250; EP 1038001 A2 Based on WO 9931250; AU 749174 B Previous Publ. AU 9916577, Based on WO 9931250; BR 9813626 A Based on WO 9931250; US 6572863 B1 Cont of US 6121021, Based on WO 9931250; JP 2004509601 W Based on WO 9931250
PRAI US 1997-991773 19971216; US 2000-555834 20000719
AB WO 9931250 A UPAB: 19990819
NOVELTY - A modified **human immunodeficiency virus** (**HIV**) genome, devoid of long terminal repeats (LTRs) and where the **vpr** and tat sequences are functionally disabled and a constitutive promoter is operatively connected to the modified genome, is new.

DETAILED DESCRIPTION - A nucleic acid molecule comprises a modified **human immunodeficiency virus** (**HIV**) genome:

(a) devoid of long terminal repeats (LTRs);

(b) functionally disabled **vpr** and tat sequences; and

(c) a constitutive promoter operatively connected to the modified genome for constitutive expression of the modified genome to produce non-infectious, non-replicating and immunogenic **HIV**-like particles.

INDEPENDENT CLAIMS are also included for:

(1) an expression vector comprising the nucleic acid as above;

(2) a method of obtaining a non-infectious, non-replication, immunogenic **HIV**-like particle; and

(3) a non-infectious, non-replicating immunogenic **HIV**-like particle lacking Tat and **Vpr** and producible by a method as above.

ACTIVITY - Anti-**HIV**.

MECHANISM OF ACTION - Vaccine.

non-infectious, non-replicating, immunogenic **HIV**-like particles are obtained by transforming a mammalian cell with a vector containing the modified **HIV** genome. The nucleic acid is constitutively expressed in the cells to stably produce the **HIV**-like particles. The **HIV**-like particles are useful as medicaments, especially for immunization (claimed). The **HIV**-like particles are useful as antigens and immunogens in the generation of anti-retroviral **antibodies** for diagnostic assays. The particles and related **antibodies** are useful in the treatment and diagnosis of AIDS and related conditions.

ADVANTAGE - The modified **HIV** genome is induced to effect long term constitutive expression of non-infectious, non-replicating, immunogenic **HIV**-like particles without causing any toxic effect on the mammalian cells expressing the particles. The production of these particles is more useful in commercial production of **HIV**-like particles than previous systems. The **HIV**-like particles comprise gag and env gene products in their native conformations. These retrovirus particles will therefore be recognized by conformational anti-**HIV antibodies** that may not recognize the **HIV** antigen in a denatured form or a synthetic peptide corresponding to such an **HIV** antigen.

DESCRIPTION OF DRAWING(S) - Expression plasmid pCMVgDtat-**vpr**-/ Dwg.2A/3

L21 ANSWER 36 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1999-243944 [20] WPIDS
DNC C1999-071160
TI New lipid-containing vector with a mutant hemagglutinin, useful in gene therapy.
DC B04 D16
IN BATES, P; MIR-SHEKARI, Y
PA (UYPE-N) UNIV PENNSYLVANIA
CYC 22
PI WO 9913905 A1 19990325 (199920)* EN 56
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP US
AU 9893994 A 19990405 (199933)
US 6416997 B1 20020709 (200253)
ADT WO 9913905 A1 WO 1998-US19552 19980917; AU 9893994 A AU 1998-93994 19980917; US 6416997 B1 Provisional US 1997-59239P 19970918, Cont of WO 1998-US19552 19980917, US 2000-525392 20000315
FDT AU 9893994 A Based on WO 9913905
PRAI US 1997-59239P 19970918; US 2000-525392 20000315
AB WO 9913905 A UPAB: 19990525
NOVELTY - A lipid-containing vector (I) capable of fusing to a cell membrane.

DETAILED DESCRIPTION - The vector comprises hemagglutinin with a mutation in the receptor binding pocket, abrogating binding to a sialic acid containing receptor but not affecting fusogenic capacity of the hemagglutinin.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of producing a vector (II) comprising pseudotyping an enveloped virus with a mutant influenza A virus hemagglutinin which comprises at least one amino acid substitution at residues threonine-115, glutamin-190 and leucine-226 in the receptor binding pocket, and where the substitution abrogates binding of the hemagglutinin to a sialic acid containing receptor, and co-pseudotyping the virus with a targeting molecule.

(2) an isolated influenza A virus hemagglutinin (III) comprising a mutation which abrogates binding to a sialic acid containing receptor, but does not affect the fusogenic capability of hemagglutinin;

(3) DNA encoding an influenza A virus hemagglutinin with a mutation in the receptor binding pocket which abrogates binding to a sialic acid receptor, but does not affect fusogenic capabilities of the hemagglutinin;

(4) a pseudotyped murine leukemia virus (MLV) (IV) comprising a mutant influenza A virus hemagglutinin, the mutation comprising a change from threonine to serine at amino acid 155, and a change from leucine to

pseudotyped MLV;

(5) a composition (V) comprising a co-pseudotyped enveloped virus expressing a mutant hemagglutinin and a targeting molecule, the co-pseudotyped virus binding to a target cell expressing a receptor for the targeting molecule, the hemagglutinin causing the virus to fuse with the cell; and

(6) mammalian cells comprising the pseudotyped MLV virus, or the co-pseudotyped virus (V).

USE - The new vectors are useful for targeted delivery of a component to a desired cell i.e. a nucleic acid, an antisense nucleic acid, a gene, a protein, a peptide, a **Vpr** protein, an enzyme, an intracellular antagonist of **HIV**, a radionuclide, a cytotoxic compound, an antiviral agent or an imaging agent (claimed) (i.e. gene therapy).

A cell-cell fusion assay between mutant and wild-type hemagglutinin showed that the new mutant was able to fuse with cells at the same levels as the wild-type, even though the receptor binding was abolished.

ADVANTAGE - Infectious titres of prior art retroviral vectors are low, and do not have an agent capable of inducing fusion of the virion envelope with the target cell membrane.

Dwg.0/12

L21 ANSWER 37 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1998-568304 [48] WPIDS
DNN N1998-442130 DNC C1998-170771
TI New nucleic acid encoding mutant or truncated forms of human immune deficiency virus proteins - used to generate non-infectious particles useful as therapeutic or prophylactic immunogens, also for diagnosis.
DC B04 D16 S03
IN LUFTIG, R B
PA (IMMU-N) IMMUNE RESPONSE CORP; (LOUU) UNIV LOUISIANA STATE
CYC 82
PI WO 9844945 A1 19981015 (199848)* EN 65
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW
AU 9871017 A 19981030 (199911)
US 6328976 B1 20011211 (200204)
US 2002061313 A1 20020523 (200239)
US 6557296 B2 20030506 (200338)
ADT WO 9844945 A1 WO 1998-US6690 19980403; AU 9871017 A AU 1998-71017 19980403; US 6328976 B1 Provisional US 1997-43047P 19970404, US 1998-55075 19980403; US 2002061313 A1 Provisional US 1997-43047P 19970404, Cont of US 1998-55075 19980403, US 2001-919124 20010730; US 6557296 B2 Provisional US 1997-43047P 19970404, Cont of US 1998-55075 19980403, US 2001-919124 20010730
FDT AU 9871017 A Based on WO 9844945; US 6557296 B2 Cont of US 6328976
PRAI US 1997-43047P 19970404; US 1998-55075 19980403; US 2001-919124 20010730
AB WO 9844945 A UPAB: 19981203
New nucleic acid (I) or its fragments encodes a truncated Nef protein (II) having substantially amino acid (aa) residues 1-56 of a **human immunodeficiency virus**-1 (**HIV**-1) Nef protein.Also new are (1) nucleic acids (Ia) encoding specific mutant Nef proteins; a mutated **HIV**-1 env gp41 protein having Arg at position 660, or its fragments with G at nucleotide position 1979; a mutated **HIV**-1 env gp120 protein in which (a) aa 143 is Ser or Arg, up to 8 aa have been deleted between 144 and 151 in the V1 domain and aa 153 is Met or Ile, or (b) aa 187 is Ile or Val, 1 or 2 aa have been deleted at 192 and 193 in the V2 domain, or its fragments containing the specified deletions; a truncated **HIV**-1 **Vpr** protein comprising aa 1-17 of **Vpr**; or mutated or truncated **HIV**-1 Pol protein comprising aa 1-13 of wild-type Pol and aa 14-29 of the sequence

**antibodies** (Ab) or their fragments that react specifically with (II).

USE - Inactive, protease-deficient **HIV**-1 particles (VP) containing at least one of (II) are used as immunogens, particularly for reducing or preventing apoptosis in **HIV**-1 sero-negative or -positive subjects, specifically those with **HIV**-1 infection, both for prevention and treatmentragments of (I) and (Ia) are also useful in hybridisation tests for diagnostic detection of mutated genes in (lysed) cells or body fluids, while the corresponding mutant proteins are detected in immunoassays using Ab. VP and Ab, optionally attached to a radioisotope, chemotherapeutic agent or toxin, can be used to reduce the severity of **HIV** infections. (II), or their fragments, are used to raise Ab.

ADVANTAGE - The specified mutations result in **HIV**-1 particles that are non-infectious.

Dwg.0/4

L21 ANSWER 38 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1998-447229 [38] WPIDS
DNN N1998-348562 DNC C1998-135742
TI Arresting cell growth using lentivirus **Vpr** virion protein - used for treatment of cancer and screening for agents that reduce **Vpr** binding, e.g. anti-**HIV** agents.
DC B04 D16 S03
IN CHEN, I S Y; DIECKMANN, T; FEIGON, J; JOWETT, J B M; POGH, B; STEWART, S A; WITHERS-WARD, E; POON, B
PA (REGC) UNIV CALIFORNIA
CYC 20
PI WO 9835032 A2 19980813 (199838)* EN 70
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP
AU 9863335 A 19980826 (199902)
ADT WO 9835032 A2 WO 1998-US3390 19980211; AU 9863335 A AU 1998-63335 19980211
FDT AU 9863335 A Based on WO 9835032
PRAI US 1997-959279 19971024; US 1997-798597 19970211
AB WO 9835032 A UPAB: 19981125
Growth of a cell is arrested by treatment with a **Vpr** lentivirus protein or its analogue.

Also claimed are:

(1) a method for identifying antitumour agents using a cellular target (II) for **Vpr**;

(2) an isolated nucleic acid (III) that encodes proteins B29-1 or B251-1, or their allelic variants;

(3) proteins (A) defined in (2);

(4) **antibodies** (Ab) that bind selectively to (A), and

(5) compounds (B) that restore growth to cells where this has been arrested by binding of **Vpr** to (II).

USE - Agents that reduce binding of **Vpr** to (II) are useful for treating **HIV** (human immune deficiency virus) infection or more generally for restoring growth.

(I) is useful for treating:

(a) any type of cancer, since it induces cell stasis (blocks development at the G2 stage) and death, and

(b) autoimmune disease.

(III) can also be used to generate knock-out transgenic animals, used to identify:

(i) compounds that mimic these proteins,

(ii) other genes and proteins that interact with them,

(iii) agents that compensate for a protein deficiency,

(iv) mutations that alter activity, and the promoters of the B29-1 or B251-1 genes are used for directing gene expression.

(I) are administered by injection or orally, typically at 0.1-100, particularly 0.1-1, mu g/kg, or they are expressed from gene therapy vectors.

Dwg.0/13

Full Text
AN 1997-538622 [50] WPIDS
CR 1990-378039 [51]
DNN N1997-448251 DNC C1997-172371
TI Oligo-nucleotide primers for amplifying retroviral nucleic acids - comprising conserved sequences of **human immunodeficiency virus** and simian immunodeficiency virus genes.
DC B04 D16 S03
IN MONCANY, M; MONTAGNIER, L; LUC, M; MAURICE, M
PA (INRM) INSERM INST NAT SANTE & RECH MEDICALE; (INSP) INST PASTEUR; (INRM) INSERM INST NAT SANTE RE; (INRM) INST NAT SANTE & RECH MEDICALE
CYC 17
PI EP 806484 A2 19971112 (199750)* FR 23
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
SG 47868 A1 19980417 (199827)
US 5786177 A 19980728 (199837)
EP 403333 B1 19991006 (199946) FR
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
DE 69033311 E 19991111 (199954)
ES 2139567 T3 20000216 (200016)
JP 2000093187 A 20000404 (200027) 14
US 6194142 B1 20010227 (200114)
JP 3428012 B2 20030722 (200350) 23
ADT EP 806484 A2 Div ex EP 1990-401520 19900605, EP 1997-110543 19900605; SG 47868 A1 SG 1996-4845 19900605; US 5786177 A Cont of US 1992-820599 19920121, Div ex US 1993-160465 19931202, US 1997-895231 19970716; EP 403333 B1 EP 1990-401520 19900605, Related to EP 1997-110543 19900605; DE 69033311 E DE 1990-633311 19900605, EP 1990-401520 19900605; ES 2139567 T3 EP 1990-401520 19900605; JP 2000093187 A Div ex JP 1990-508911 19900605, JP 1999-270165 19900605; US 6194142 B1 Cont of US 1992-820599 19920121, Div ex US 1993-160465 19931202, Div ex US 1997-895231 19970716, US 1998-92077 19980605; JP 3428012 B2 JP 1990-508911 19900605, WO 1990-FR393 19900605
FDT EP 806484 A2 Div ex EP 403333; US 5786177 A Div ex US 5688637; EP 403333 B1 Related to EP 806484; DE 69033311 E Based on EP 403333; ES 2139567 T3 Based on EP 403333; US 6194142 B1 Div ex US 5688637, Div ex US 5786177; JP 3428012 B2 Previous Publ. JP 04507043, Based on WO 9015066
PRAI FR 1989-12371 19890920; FR 1989-7354 19890602; WO 1990-FR393 19900506
AB EP 806484 A UPAB: 20040418
Oligonucleotides useful as primers for nucleic acid amplification comprise conserved sequences of the gag, **vpr**, pol or vpu genes of **HIV**-1 Bru, **HIV**-1 Mal, **HIV**-1 Eli, **HIV**-2 ROD and SIV MAC or the nef2, vif2 or vpx genes of **HIV**-2 ROD and SIV MAC.
USE - The amplification method and **antibodies** are useful for diagnosis of **HIV**-1, **HIV**-2 or SIV infections.
Dwg.0/0

L21 ANSWER 40 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1995-231517 [30] WPIDS
DNC C1995-106867
TI Pure **vpr** receptor protein - useful to treat individuals exposed to **HIV** and to identify anti-**HIV** agents that inhibit binding of **vpr** to the receptor.
DC B04
IN LEVY, D N; REFAELI, Y; WEINER, D B
PA (UYPE-N) UNIV PENNSYLVANIA
CYC 20
PI WO 9516705 A1 19950622 (199530)* EN 31
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU CA JP US
AU 9513733 A 19950703 (199542)
US 5780238 A 19980714 (199835)
ADT WO 9516705 A1 WO 1994-US14532 19941215; AU 9513733 A AU 1995-13733

19941215, US 1996-652572 19961024
FDT AU 9513733 A Based on WO 9516705; US 5780238 A Based on WO 9516705
PRAI US 1993-167519 19931215; US 1996-652572 19961024
AB WO 9516705 A UPAB: 19950804
Pure **vpr** receptor protein (I) with mol. wt. of 41 kD (as determined by12% SDS-PAGE) which binds **vpr** and is soluble in Triton, or a fragment binding **vpr** are claimed. Also claimed are: (1) identification of cpds. that inhibit binding of **vpr** to (I) by: (a) contacting the cpd. with **vpr** or a fragment and (I) or a fragment; (b) determining the level of binding; and (c) comparing the level of binding to that occurring when **vpr** and (I) are contacted in the absence of the cpd.; (2) a kit for identifying cpds. that inhibits binding of **vpr** to (I) comprising: (a) a first container comprising **vpr** protein or a fragment, and (b) a second container comprising (I) or a fragment; and (c) isolated **antibodies** which specifically bind to (I); and (3) isolated **antibodies** that bind specifically to the **vpr** protein of mol. wt. 41 kD as above.

USE - (I), **vpr** or fragments can be used to treat individual exposed to **HIV** (claimed). The method can be used to identify cpds. that inhibit binding of **vpr** to (I). These cpds. are useful to impede **HIV** replication and so would be useful as anti-**HIV** agents alone or as part of a multifaceted anti-**HIV** drug regimen. The **antibodies** may be used for immunoassays, e.g. bound to a solid support, to detect or quantitate **vpr** binding to (I).
Dwg.0/0

L21 ANSWER 41 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1994-294323 [36] WPIDS
DNC C1994-134441
TI Use of **viral protein R** and nucleic acid - for inducing differentiation of cells and in screening assays, diagnosis and therapy of e.g. **HIV** infection.
DC B04 D16
IN LEVY, D N; REFAELI, Y; WEINER, D B
PA (WEIN-I) WEINER D B; (LEVY-I) LEVY D N; (REFA-I) REFAELI Y; (LEVY-I) LEVY M; (UYPE-N) UNIV PENNSYLVANIA; (WIST-N) WISTAR INST
CYC 50
PI WO 9419456 A1 19940901 (199436)* EN 114
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE
W: AT AU BB BG BR BY CA CH CN CZ DE DK ES FI GB HU JP KP KR KZ LK LU LV MG MN MW NL NO NZ PL PT RO RU SD SE SK UA US UZ VN
AU 9462524 A 19940914 (199502)
ZA 9401111 A 19941130 (199503) 32
EP 689586 A1 19960103 (199606) EN
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
EP 689586 A4 19971229 (199840)
US 5874225 A 19990223 (199915)
IL 108707 A 19990620 (199937)
US 2003207252 A1 20031106 (200374)
ADT WO 9419456 A1 WO 1994-US2191 19940222; AU 9462524 A AU 1994-62524 19940222; ZA 9401111 A ZA 1994-1111 19940218; EP 689586 A1 EP 1994-909839 19940222, WO 1994-US2191 19940222; EP 689586 A4 EP 1994-909839 19940222; US 5874225 A US 1993-19601 19930219; IL 108707 A IL 1994-108707 19940218; US 2003207252 A1 CIP of US 1993-19601 19930219, Div ex US 1993-167608 19931215, US 2001-935100 20010822
FDT AU 9462524 A Based on WO 9419456; EP 689586 A1 Based on WO 9419456; US 2003207252 A1 CIP of US 5874225
PRAI US 1993-167608 19931215; US 1993-19601 19930219; US 2001-935100 20010822
AB WO 9419456 A UPAB: 19981021
(A) A method of inducing undifferentiated cells to differentiate comprises (a) contacting undifferentiated cells with **viral protein R** (**vpr**) or a functional fragment effective to stimulate differentiation, or (b) introducing into undifferentiated cells a nucleic acid molecule that comprises a nucleotide sequence that encodes **vpr** or a functional

fragment whereby the nucleotide sequence is expressed by the cells.

USE - The **vpr** or nucleic acid can be used for treating a disease characterised by hyper-proliferating undifferentiated cells, a disease associated with the loss or dysfunction of cells or a disease characterised by undesirable activity of macrophage cells (claimed). The **vpr** and **antibodies** to it can be used for identifying individuals exposed to **HIV** and for treating individuals exposed to **HIV** (claimed). The prods. can also be used to identify inhibitory cpds. and in diagnosis and therapy in diseases such as, e.g. cancer, psoriasis, autoimmune disease, granuloma or Parkinson's disease.

Dwg.0/0

L21 ANSWER 42 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1991-022239 [03] WPIDS
DNC C1991-009577
TI Vector contg. active **viral protein R** gene encoding protein - having ability to trans-activate, useful for producing high levels of desired heterologous gene prod..
DC B04 D16
IN COHEN, E; HASELTINE, W A; TERWILLIGE, E; TERWILLIGER, E
PA (DAND) DANA FARBER CANCER INST INC
CYC 15
PI WO 9015875 A 19901227 (199103)*
RW: AT BE CH DE DK ES FR GB IT LU NL SE
W: CA JP
EP 474797 A 19920318 (199212) 39
R: AT BE CH DE DK ES FR GB IT LI LU
JP 04506605 W 19921119 (199301) 14
EP 474797 A4 19920805 (199523)
EP 474797 B1 19950906 (199540) EN
R: AT BE CH DE DK ES FR GB IT LI LU NL SE
DE 69022233 E 19951012 (199546)
ADT EP 474797 A EP 1990-915880 19900601; JP 04506605 W JP 1990-514932 19900601, WO 1990-US3126 19900601; EP 474797 A4 EP 1990-915880 ; EP 474797 B1 EP 1990-915880 19900601, WO 1990-US3126 19900601; DE 69022233 E DE 1990-622233 19900601, EP 1990-915880 19900601, WO 1990-US3126 19900601
FDT JP 04506605 W Based on WO 9015875; EP 474797 B1 Based on WO 9015875; DE 69022233 E Based on EP 474797, Based on WO 9015875
PRAI US 1989-361028 19890602
AB WO 9015875 A UPAB: 19930928
A vector contg. (a) a nucleotide segment (I) contg. a sufficient number of nucleotides corresp. to an active **vpr** gene of an **HIV** genome to express a protein having the ability to trans activate; and (b) a promoter upstream of (I) is new.

Also claimed are (1) a DNA segment contg. essentially of an active **vpr** gene, but not the entire **HIV** genome; (2) a pure recombinant protein (mol.wt. ca. 15 kD) produced by the DNA of (1); (3) a protein produced by (I); (4) an **antibody** (Ab) specifically reacting with the protein of (2); (5) an immunogenic oligopeptide corresp. to a sufficient number of amino acids from the C terminal portion of the protein of (2) to produce an Ab; (6) an Ab generated by the oligopeptide of (5); (7) an assay for the presence of active **viral protein R** by (i) taking a predetermined sample, (ii) adding the Ab of (6), and (iii) determining whether a complex is formed with the Ab; (8) a cell line transformed by the vector; and (9) a method of producing high levels of a desired heterologous gene prod.

USE/ADVANTAGE - The functional **vpr** protein has the ability to accelerate growth in trans, thus the **vpr** vector can be used to increase the prodn. of a desired heterologous gene prod., e.g. the chloramphenical acetyltransferase (CAT) gene prod. @(39pp Dwg.No.0/13)

L21 ANSWER 43 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1990-378039 [51] WPIDS

DNN N1990-288105 DNC C1990-164681
TI New nucleotide sequences derived from genome of **HIV**-1, **HIV**-2 and SIV - useful as primers for amplification of immuno-deficiency viruses in diagnosis and for raising **antibodies** in treatment of **HIV** infections.
DC B04 D16
IN MONCANY, M; MONTAGNIER, L
PA (INRM) INSERM INST NAT SANTE RE; (INSP) INST PASTEUR; (INRM) INSERM INST NAT SANTE & RECH MEDICALE; (INRM) INST NAT SANTE & RECH MEDICALE
CYC 17
PI EP 403333 A 19901219 (199051)* 24
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
WO 9015066 A 19901213 (199101)
W: CA JP US
FR 2647809 A 19901207 (199105)#
FR 2652091 A 19910322 (199121)
JP 04507043 W 19921210 (199304)# 15
WO 9015066 A3 19910418 (199508)
US 5688637 A 19971118 (199801) 12
EP 403333 B1 19991006 (199946) FR
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
ES 2139567 T3 20000216 (200016)
JP 2000093187 A 20000404 (200027) 14
JP 3428012 B2 20030722 (200350) 23
ADT EP 403333 A EP 1990-401520 19900605; FR 2647809 A FR 1989-7354 19890602; FR 2652091 A FR 1989-12371 19890920; JP 04507043 W JP 1990-508911 19900605, WO 1990-FR393 19900605; WO 9015066 A3 WO 1990-FR393 19900605; US 5688637 A Cont of WO 1990-FR393 19900605, Cont of US 1992-820599 19920121, US 1993-160465 19931202; EP 403333 B1 EP 1990-401520 19900605, Related to EP 1997-110543 19900605; ES 2139567 T3 EP 1990-401520 19900605; JP 2000093187 A Div ex JP 1990-508911 19900605, JP 1999-270165 19900605; JP 3428012 B2 JP 1990-508911 19900605, WO 1990-FR393 19900605
FDT JP 04507043 W Based on WO 9015066; EP 403333 B1 Related to EP 806484; ES 2139567 T3 Based on EP 403333; JP 3428012 B2 Previous Publ. JP 04507043, Based on WO 9015066
PRAI FR 1989-12371 19890920; FR 1989-7354 19890602; JP 1990-508911 19900605
AB EP 403333 A UPAB: 20030805
New nucleotide sequences (I) are: (1) sequences present within the gag, **vpr**, pol, env, nef1 or vif1 genes of **HIV**-1 Bru, **HIV**-1 Mal or **HIV**-1 Eli, or within the gag, **vpr**, pol, nef2, vif2 or vpx genes of **HIV**-2 ROD or SIV MAC, or (2), esp. for larger primers, they contain the gene sequences specified in (1), or a complementary sequence, and the opt. additional nucleotides which flank this sequence are pref. those naturally present in the complete viral sequence, or (3) if the sequence of (I) is not identical to one of the sequences (or its complement) described above, it is nevertheless able to hybridise with a nucleic acid sequence derived from the specified viruses.

Also new are (1) immunogens contg. at least one translation prod. (II) of opt. amplified (I), and (2) **antibodies** (Ab) against (II).

USE/ADVANTAGE - (I) are useful as primers for amplifying specific viral sequences, esp. in conjunction with in vitro diagnosis of infection. They provide amplification of all strains of a given virus, with max yields and without formation of numerous non-specific bands. Anti-sense nucleotide sequences (I) can be used to treat virus diseases (esp. AIDS) and Ab are useful as immunoassay reagents.

Dwg.0/0

L21 ANSWER 44 OF 44 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 1987-221261 [31] WPIDS
CR 1987-329355 [47]; 1988-149264 [22]; 1988-220290 [31]; 1988-272808 [39]; 1992-041067 [05]; 2000-328365 [28]; 2002-434814 [46]; 2003-553960 [52]; 2004-070575 [07]
DNN N1987-165620 DNC C1987-093098
TI New type of human immuno-deficiency virus, infections for T4 cells - and

derived antigens, immunogens, monoclonal antibodies and nucleic acid sequences, e.g. for diagnosis of AIDS.

DC B04 D16 S03

IN ALIZON, M; BRUN-VEZINET, F; CHAMARET, S; CLAVEL, F; GUETARD, D; GUYADER, M; KATLAMA, C; MONTAGNIER, L; REY, M; ROUZIOUX, C; SONIGO, P; BRUNVEZINET, F; ROUXIOUX, C; SOLANGE, C; GEUTARD, D; DUETARD, D; GUETARD, D G; BURNVEZINE, F

PA (INSP) INST PASTEUR; (ALIZ-I) ALIZON M; (BRUN-I) BRUN-VEZINET F; (CLAV-I) CLAVEL F; (GUET-I) GUETARD D; (MONT-I) MONTAGNIER L

CYC 24

PI WO 8704459 A 19870730 (198731)* FR 116
RW: OA
W: AU DK JP KR US
FR 2593189 A 19870724 (198736)
FR 2593190 A 19870724 (198736)
FR 2593922 A 19870807 (198738)
EP 239425 A 19870930 (198739) FR 164
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
FR 2594229 A 19870814 (198739)
AU 8768911 A 19870814 (198743)
FR 2596063 A 19870925 (198743)
ZA 8700469 A 19870714 (198744)
FR 2597500 A 19871023 (198750)
PT 84182 A 19880122 (198809)
DK 8704934 A 19871117 (198827)
JP 63502242 W 19880901 (198841)
EP 320495 A 19890614 (198924) FR
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
US 4839288 A 19890613 (198930) 8
EP 239425 B 19891102 (198944) FR
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
DE 3760912 G 19891207 (198950)
ES 2013295 B 19900501 (199023)
US 5030718 A 19910709 (199130)
US 5051496 A 19910924 (199141)
US 5055391 A 19911008 (199143)
US 5066782 A 19911119 (199149)
US 5268265 A 19931207 (199350)#
US 5306614 A 19940426 (199416) 32
US 5310651 A 19940510 (199418) 15
JP 06113833 A 19940426 (199421) 34
US 5364933 A 19941115 (199445) 7
JP 07233196 A 19950905 (199544) 27
JP 08113598 A 19960507 (199628) 28
CA 1338323 C 19960514 (199629)
US 5545726 A 19960813 (199638) 14
JP 08275783 A 19961022 (199701) 29
US 5578715 A 19961126 (199702) 18
US 5580739 A 19961203 (199703) 42
US 5597896 A 19970128 (199710) 7
JP 09037778 A 19970210 (199716) 27
JP 09075092 A 19970325 (199722) 27
JP 2611106 B2 19970521 (199725) 48
JP 2735521 B2 19980402 (199818) 28
JP 2771519 B2 19980702 (199831) 27
US 5770703 A 19980623 (199832)
JP 2801162 B2 19980921 (199843) 30
MX 185326 A 19970718 (199846)
US 5830641 A 19981103 (199851)
US 5866319 A 19990202 (199912)
JP 11018768 A 19990126 (199914) 29
JP 2865203 B2 19990308 (199915) 33
JP 2874846 B2 19990324 (199917) 33
US 5889158 A 19990330 (199920)
JP 2931294 B2 19990809 (199937) 28
US 5976785 A 19991102 (199953)

US 6048685 A 20000411 (200025)
EP 320495 B1 20000802 (200038)# FR
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
DE 3752319 G 20000907 (200044)
US 6162439 A 20001219 (200102)
ES 2150897 T3 20001216 (200105)
US 6261762 B1 20010717 (200142)
US 6265149 B1 20010724 (200146)
US 6296807 B1 20011002 (200160)
US 6316183 B1 20011113 (200173)
US 6355789 B1 20020312 (200221)
US 6429306 B1 20020806 (200254)
US 6514691 B1 20030204 (200313)
US 6518015 B1 20030211 (200314)
US 2003082523 A1 20030501 (200331)
US 2003091985 A1 20030515 (200335)
US 2003186219 A1 20031002 (200365)
US 2003170658 A1 20030911 (200367)
IE 83207 B 20031210 (200381)
US 6664041 B2 20031216 (200382)

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(+24.3.86, 18.3.86, 13.2.86, 6.2.86, 22.1.86-FR-004215, 003881, 001985, 001635, 000911)

New **HIV**-2 (human innunodeficiency virus), or its variants, are infectious for human T4 lymphocytes and have the morphological and immunological characteristics of the viruses deposited as I-502, -532, -642, and -643 (CNCM).

Also new are (1) antigens (Ag), forming a single bond in polyacrylamide gel electrophoresis, contg. an epitope recognised by the serum of a carrier of **HIV**-2 **antibodies**; (2) immunogens contg. **HIV**-2 glycoprotein; (3) monoclonal antibdoies (MAb) which specifically recognise (Ag; (4) the hybridomas which produce MAb; and (5) nucleic acid sequences, opt. labelled, derived from at least part of the RNA of **HIV**-2, or its variants.

USE/ADVANTAGE - Ag are useful for detecting presence of anti-**HIV**-2 **antibodies** in the serum, esp. for diagnosis of AIDS. The virus itself, or its RNA, can be detected by using the nucleic acid sequences as hybridisation probes. The immunogens are useful for making vaccines against **HIV**-2.

Dwg.0/8

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(FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
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L3 26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4 26 S L3 AND ANTIBOD?
E LEVY DAVID/IN
L5 23 S E3
L6 23 S L5 NOT L4
E REFAELI YOSEF/IN
L7 6 S E3
L8 0 S L7 NOT (L5 OR L1)
E MONTAGNIER LUC/IN
L9 99 S E3
L10 9 S L9 AND (VPR OR VIRAL PROTEIN R)
L11 33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L12 594 S L11 AND (VPR OR VIRAL PROTEIN R)
L13 548 S L12 AND ANTIBOD?
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L15 35 S L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)
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L17 9 S E3 OR E4
L18 2 S L17 AND (VPR OR VIRAL PROTEIN R)

FILE 'WPIDS' ENTERED AT 08:14:50 ON 28 JUN 2004
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L21 44 S L20 AND ANTIBOD?

=> file medline

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 179.40 | 419.64 |

FILE 'MEDLINE' ENTERED AT 08:19:47 ON 28 JUN 2004

FILE LAST UPDATED: 26 JUN 2004 (20040626/UP). FILE COVERS 1951 TO DATE.

On February 29, 2004, the 2004 MeSH terms were loaded. See HELP RLOAD for details. OLDMEDLINE now back to 1951.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a description of changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

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L24  ANSWER 1 OF 3     MEDLINE on STN
2001183372.    PubMed ID: 11244567.   Choice of expression vector alters the
     localization of a human cellular protein. Ramanathan M P; Ayyavoo V;
     Weiner D B. (Department of Pathology & Laboratory Medicine, University
     of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA. ) DNA
     and cell biology, (2001 Feb) 20 (2) 101-5.  Journal code: 9004522. ISSN:
     1044-5498. Pub. country: United States. Language: English.
AB   The fusion of synthetic epitopes with proteins of interest is an important
     tool in the identification and characterization of recombinant proteins.
     Several mammalian expression vectors are commercially available containing
     unique identification tags or epitopes.  These vectors offer a great
     advantage to researchers, as highly specific antibodies and purification
     resins against these specific epitopes are readily available.  The tags
     facilitate immunologic assays and the purification of the recombinant
     proteins.  The fusion of these epitopes with the recombinant proteins is
     not expected to alter the behavior of the protein of interest.  In this
     report, we demonstrate that the mere expression of a cellular protein,
     hVIP/mov34, which we earlier identified as a cellular HIV-1 Vpr ligand,
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in the different vectors clearly affected its localization pattern in HeLa cells. Specifically, cloning of hVIP/mov34 in pcDNA3/HisA resulted in its nuclear localization, whereas the expression of this gene from a TOPO cloning expression vector, pcDNA3.1/V5/His, resulted in cytoplasmic expression. The native staining pattern of hVIP/mov34 using polyclonal antisera raised against hVIP/mov34 demonstrated cytoplasmic staining. During cloning, other leader sequences intended for targeting this protein into a cytoplasmic or a nuclear location were not fused to the actual ORF of this protein. Also, the amino acid sequence of the fusion region arising from cloning of hVIP/mov34 in both vectors does not match any reported NLS sequences. These results indicate that the choice of the expression vectors, as well as the position of synthetic epitopes, can significantly alter the behavior and the biology of recombinant proteins. This result suggests the need for a careful examination of these features when characterizing a newly identified protein.

L24 ANSWER 2 OF 3 MEDLINE on STN

95115082. PubMed ID: 7815499. Extracellular **Vpr** protein increases cellular permissiveness to human immunodeficiency virus replication and reactivates virus from latency. Levy D N; Refaeli Y; **Weiner D B.** (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104-4283. ) Journal of virology, (1995 Feb) 69 (2) 1243-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The **vpr** gene product of human immunodeficiency virus (HIV) and simian immunodeficiency virus is a virion-associated regulatory protein that has been shown using **vpr** mutant viruses to increase virus replication, particularly in monocytes/macrophages. We have previously shown that **vpr** can directly inhibit cell proliferation and induce cell differentiation, events linked to the control of HIV replication, and also that the replication of a **vpr** mutant but not that of wild-type HIV type 1 (HIV-1) was compatible with cellular proliferation (D. N. Levy, L. S. Fernandes, W. V. Williams, and D. B. Weiner, Cell 72:541-550, 1993). Here we show that purified recombinant **Vpr** protein, in concentrations of < 100 pg/ml to 100 ng/ml, increases wild-type HIV-1 replication in newly infected transformed cell lines via a long-lasting increase in cellular permissiveness to HIV replication. The activity of extracellular **Vpr** protein could be completely inhibited by anti-**Vpr antibodies**. Extracellular **Vpr** also induced efficient HIV-1 replication in newly infected resting peripheral blood mononuclear cells. Extracellular **Vpr** transcomplemented a **vpr** mutant virus which was deficient in replication in promonocytic cells, restoring full replication competence. In addition, extracellular **Vpr** reactivated HIV-1 expression in five latently infected cell lines of T-cell, B-cell, and promonocytic origin which normally express very low levels of HIV RNA and protein, indicating an activation of translational or pretranslational events in the virus life cycle. Together, these results describe a novel pathway governing HIV replication and a potential target for the development of anti-HIV therapeutics.

L24 ANSWER 3 OF 3 MEDLINE on STN

95062167. PubMed ID: 7971975. Serum **Vpr** regulates productive infection and latency of human immunodeficiency virus type 1. Levy D N; Refaeli Y; MacGregor R R; **Weiner D B.** (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104. ) Proceedings of the National Academy of Sciences of the United States of America, (1994 Nov 8) 91 (23) 10873-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB In human immunodeficiency virus (HIV)-positive individuals, the vast majority of infected peripheral blood cells and lymph node cells may be latently or nonproductively infected. The **vpr** open reading frame of HIV-1 encodes a 15-kDa virion-associated protein, **Vpr**. The **vpr** gene has been shown to increase virus replication in T cells and monocyte/macrophages in vitro. We have previously reported that **vpr** expression in various tumor lines leads to growth inhibition and differentiation, indicating that **Vpr** may function as a regulator of

protein is present in significant amounts in the serum of AIDS patients. Purified serum **Vpr** activated virus expression from five latently infected cell lines, U1, OM.10.1, ACH-2, J1.1, and LL58. Serum **Vpr** also activated virus expression from resting peripheral blood mononuclear cells of HIV-infected individuals. Together, these findings implicate serum **Vpr** in the activation of HIV replication in vivo and in the control of latency. Anti-**Vpr antibodies** inhibited **Vpr** activity, suggesting that humoral immunity modulates **Vpr** activity in vivo. These results have broad implications for the virus life cycle and for the prospective control of HIV replication and pathogenesis.

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(FILE 'HOME' ENTERED AT 07:44:30 ON 28 JUN 2004)

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L1 40 S E5
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L3 26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4 26 S L3 AND ANTIBOD?
E LEVY DAVID/IN
L5 23 S E3
L6 23 S L5 NOT L4
E REFAELI YOSEF/IN
L7 6 S E3
L8 0 S L7 NOT (L5 OR L1)
E MONTAGNIER LUC/IN
L9 99 S E3
L10 9 S L9 AND (VPR OR VIRAL PROTEIN R)
L11 33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
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L13 548 S L12 AND ANTIBOD?
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L24 3 S L23 AND ANTIBOD?

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9697 HTLV
211040 III
1640 HTLV-III
(HTLV(W)III)
8571542 HUMAN
113598 IMMUNODEFICIENCY
376647 VIRUS
43126 HUMAN IMMUNODEFICIENCY VIRUS
(HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
8571542 HUMAN
3684614 T

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        173822 LEUKEMIA
        376647 VIRUS
        783437 TYPE
        211040 III
            15 HUMAN T-CELL LEUKEMIA VIRUS TYPE III
                 (HUMAN(W)T(W)CELL(W)LEUKEMIA(W)VIRUS(W)TYPE(W)III)
L25     143517 (HIV OR HTLV-III OR HUMAN IMMUNODEFICIENCY VIRUS OR HUMAN T-CELL
                LEUKEMIA VIRUS TYPE III)

=> s l25 and (Vpr or viral protein R)
           763 VPR
        313682 VIRAL
       1282020 PROTEIN
        246213 R
            52 VIRAL PROTEIN R
                 (VIRAL(W)PROTEIN(W)R)
L26        623 L25 AND (VPR OR VIRAL PROTEIN R)

=> s l26 and (antibod? or antiser?)
        663895 ANTIBOD?
         55508 ANTISER?
L27         69 L26 AND (ANTIBOD? OR ANTISER?)

=> d l27,cbib,ab,1-69

L27  ANSWER 1 OF 69     MEDLINE on STN
2003542529.     PubMed ID: 14615141.   The HIV-1 chimeric protein CR3
     expressed by poxviral vectors induces a diverse CD8+ T cell response in
     mice and is antigenic for PBMCs from HIV+ patients. Vazquez-Blomquist
     Dania; Iglesias Enrique; Gonzalez-Horta Eddy E; Duarte Carlos A.
     (Departamento de SIDA, Division de Vacunas, Centro de Ingenieria Genetica
     y Biotecnologia, Apdo 6162, Cubanacan, Playa, 10600, Ciudad Habana, Cuba.
     ) Vaccine, (2003 Dec 12) 22 (2) 145-55.  Journal code: 8406899. ISSN:
     0264-410X. Pub. country: Netherlands. Language: English.
AB   Recombinant avipoxvirus vectors are attractive for vaccination against
     human immunodeficiency virus type 1 (HIV-1), where induction of a
     cytotoxic CD8(+) T cell (CTL) response seems to be an important component
     of protective immunity.  We expressed the chimeric protein CR3, composed
     by CTL epitopes rich regions from, RT, Gag and Nef and conserved Th cell
     epitopes from gp120, gp41 and Vpr of HIV-1 in a fowlpox virus (FWPV)
     vector (FPCR3), and used this vector to induce HIV-specific CTL
     responses in mice.  Mice immunised twice intraperitoneally with FPCR3,
     developed a CD8(+) T cell response measured as production of IFN-gamma by
     splenocytes in response to stimulation with P815 cells infected with
     recombinant vaccinia viruses (rVV) expressing CR3, Gag and Nef.  The
     number of IFN-gamma secreting cells was markedly higher when a P815 cell
     line constitutively expressing CR3 was used as target cells for
     Enzyme-linked-immunospot (ELISPOT).  CR3 epitopes were also specifically
     recognised by human PBMCs from three HIV(+) patients with different
     haplotypes.  These results confirm the potential of FWPV vectors
     expressing these novel HIV-1 chimeric proteins to induce a simultaneous
     CD8(+) T cell response against conserved viral targets and early expressed
     regulatory proteins.

L27  ANSWER 2 OF 69     MEDLINE on STN
2003283607.     PubMed ID: 12810859.   Construction and in vivo infection of a
     new simian/human immunodeficiency virus chimera containing the
     reverse transcriptase gene and the 3' half of the genomic region of
     human immunodeficiency virus type 1. Akiyama Hisashi; Ido Eiji;
     Akahata Wataru; Kuwata Takeo; Miura Tomoyuki; Hayami Masanori. (Institute
     for Virus Research, Laboratory of Viral Pathogenesis, Kyoto University, 53
     Shogoin-kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan. ) Journal of general
     virology, (2003 Jul) 84 (Pt 7) 1663-9.  Journal code: 0077340. ISSN:
     0022-1317. Pub. country: England: United Kingdom. Language: English.
AB   A new simian/human immunodeficiency virus (SHIV) chimera with the
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reverse transcriptase (RT) coding region of pol, in addition to the 3' region encoding **vpr**, vpu, tat, rev, env and nef of **HIV**-1, on an SIV(mac) (SIV from a macaque monkey) background was constructed. This new SHIV chimera, named SHIVrt/3rn, could replicate in monkey peripheral blood mononuclear cells (PBMCs) as well as in the human and monkey CD4(+) T-cell lines M8166 and HSC-F. Since SHIVrt/3rn contains the RT gene of **HIV**-1, replication of the virus in M8166 cells was inhibited by an **HIV**-1-specific non-nucleoside RT inhibitor, MKC-442, with a sensitivity similar to that of **HIV**-1. To investigate the replication competence of SHIVrt/3rn in vivo, two rhesus monkeys were inoculated intravenously with the virus. At 2 to 4 weeks post-inoculation (p.i.), plasma viral RNA loads of both monkeys showed a peak value of more than 10(4) copies ml(-1). Infectious virus was isolated from the PBMCs of one monkey at 2 and 3 weeks p.i. and from the other at 4 weeks p.i. Moreover, proviral DNA was detected constantly throughout the observation period, starting from 3 weeks p.i. An **antibody** response, detected first at 3 weeks p.i., was maintained at high titres. These results indicate that SHIVrt/3rn can infect and replicate in vivo. SHIVrt/3rn, having part of **HIV**-1 pol in addition to the 3' part of the **HIV**-1 genome is genetically more close to **HIV**-1 than any of the other monkey-infecting SHIVs reported previously.

L27 ANSWER 3 OF 69 MEDLINE on STN

2003245129. PubMed ID: 12768007. Characterization of a novel simian immunodeficiency virus (SIVmonNG1) genome sequence from a mona monkey (Cercopithecus mona). Barlow Katrina L; Ajao Adebowale Oluwafemi; Clewley Jonathan P. (Sexually Transmitted and Blood Borne Virus Laboratory, Central Public Health Laboratory, London NW9 5HT, United Kingdom. ) Journal of virology, (2003 Jun) 77 (12) 6879-88. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A novel simian immunodeficiency virus (SIV) sequence has been recovered from RNA extracted from the serum of a mona monkey (Cercopithecus mona) wild born in Nigeria. The sequence was obtained by using novel generic (degenerate) PCR primers and spans from two-thirds into the gag gene to the 3' poly(A) tail of the SIVmonNG1 RNA genome. Analysis of the open reading frames revealed that the SIVmonNG1 genome codes for a Vpu protein, in addition to Gag, Pol, Vif, **Vpr**, Tat, Rev, Env, and Nef proteins. Previously, only lentiviruses infecting humans (**human immunodeficiency virus** type 1 [**HIV**-1]) and chimpanzees (SIVcpz) were known to have a vpu gene; more recently, this has also been found in SIVgsn from Cercopithecus nictitans. Overall, SIVmonNG1 most closely resembles SIVgsn: the env gene sequence groups with **HIV**-1/SIVcpz env sequences, whereas the pol gene sequence clusters closely with the pol sequence of SIVsyk from Cercopithecus albogaris. By bootscanning and similarity plotting, the first half of pol resembles SIVsyk, whereas the latter part is closer to SIVcol from Colobus guereza. The similarities between the complex mosaic genomes of SIVmonNG1 and SIVgsn are consistent with a shared or common lineage. These data further highlight the intricate nature of the relationships between the SIVs from different primate species and will be helpful for unraveling these associations.

L27 ANSWER 4 OF 69 MEDLINE on STN

2003241963. PubMed ID: 12730499. Recruitment of **HIV** and its receptors to dendritic cell-T cell junctions. McDonald David; Wu Li; Bohks Stacy M; KewalRamani Vineet N; Unutmaz Derya; Hope Thomas J. (Department of Microbiology and Immunology, University of Illinois, Chicago, IL 60612, USA. ) Science, (2003 May 23) 300 (5623) 1295-7. Journal code: 0404511. ISSN: 1095-9203. Pub. country: United States. Language: English.

AB Monocyte-derived dendritic cells (MDDCs) can efficiently bind and transfer **HIV** infectivity without themselves becoming infected. Using live-cell microscopy, we found that **HIV** was recruited to sites of cell contact in MDDCs. Analysis of conjugates between MDDCs and T cells revealed that, in the absence of antigen-specific signaling, the **HIV** receptors CD4, CCR5, and CXCR4 on the T cell were recruited to the interface while the MDDCs concentrated **HIV** to the same region. We propose that contact between dendritic cells and T cells facilitates transmission of **HIV** by locally concentrating virus, receptor, and coreceptor during the formation of an

infectious synapse.

L27 ANSWER 5 OF 69 MEDLINE on STN

2003147838. PubMed ID: 12663813. The barrier-to-autointegration factor is a component of functional **human immunodeficiency virus** type 1 preintegration complexes. Lin Chou-Wen; Engelman Alan. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115, USA. ) Journal of virology, (2003 Apr) 77 (8) 5030-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Retroviral integration in vivo is mediated by preintegration complexes (PICs) derived from infectious virions. In addition to the integrase enzyme and cDNA substrate, PICs contain a variety of viral and host cell proteins. Whereas two different cell proteins, high-mobility group protein A1 (HMGA1) and the barrier-to-autointegration factor (BAF), were identified as integration cofactors based on activities in in vitro PIC assays, only HMGA1 was previously identified as a PIC component. By using **antibodies** against known viral and cellular PIC components, we demonstrate here functional coimmunoprecipitation of endogenous BAF protein with **human immunodeficiency virus** type 1 (**HIV**-1) PICs. Since integrase protein and integration activity were also coimmunoprecipitated by anti-BAF **antibodies**, we conclude that BAF is a component of **HIV**-1 PICs. These data are consistent with the model that BAF functions as an integration cofactor in vivo.

L27 ANSWER 6 OF 69 MEDLINE on STN

2003127552. PubMed ID: 12642031. **Antibodies** to Tat and **Vpr** in the GRIV cohort: differential association with maintenance of long-term non-progression status in **HIV**-1 infection. Richardson Max W; Mirchandani Jyotika; Duong Joseph; Grimaldo Sammy; Kocieda Virginia; Hendel Houria; Khalili Kamel; Zagury Jean Francois; Rappaport Jay. (Center for Neurovirology and Cancer Biology, 224B BLS Building, Temple University, 1900 N. 12th Street, Philadelphia, PA 19122, USA. ) Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, (2003 Jan) 57 (1) 4-14. Journal code: 8213295. ISSN: 0753-3322. Pub. country: France. Language: English.

AB The **HIV**-1 regulatory protein Tat and the accessory protein **Vpr** are thought to stimulate viral replication and contribute to viral pathogenesis as extracellular proteins. Humoral immune responses to these early viral proteins may therefore be beneficial. We examined serum anti-Tat and anti-**Vpr** IgG by ELISA in the GRIV cohort of **HIV**-1 seropositive slow/non-progressors (NP) and fast-progressors (FP), and in seronegative controls. Based on information obtained during a brief follow-up period (median = 20 months), NPs were sub-grouped as those maintaining non-progression status and therefore stable (NP-S), and those showing signs of disease progression (NP-P). As the primary comparison, initial serum anti-Tat and anti-**Vpr** IgG (prior to follow-up) were analyzed in the NP sub-groups and in FPs. Anti-Tat IgG was significantly higher in stable NP-S compared to unstable NP-P ($P = 0.047$) and FPs ($P < 0.0005$); the predictive value of higher anti-Tat IgG for maintenance of non-progression status was 92% ($P = 0.029$). In contrast, no-difference was observed in anti-**Vpr** IgG between NP-S and NP-P, although both were significantly higher than FPs ($P </= 0.001$). Serum anti-Tat IgG mapped to linear epitopes within the amino-terminus, the basic domain and the carboxy-terminal region of Tat in stable NP-S. Similar epitopes were identified in patients immunized with the Tat-toxoid in a Phase I study in Milan. High titer serum anti-Tat IgG from both GRIV and Milan cohorts cross-reacted in ELISA with Tat from diverse viral isolates, including **HIV**-1 subtype-E (CMU08) and SIVmac251 Tat; a correlation was observed between anti-Tat IgG titers and cross-reactivity. These results demonstrate that higher levels of serum anti-Tat IgG, but not anti-**Vpr** IgG, are associated with maintenance of non-progression status in **HIV**-1 infection. Evidence that vaccination with the Tat toxoid induces humoral immune responses to Tat similar to those observed in stable non-progressors is encouraging for vaccine strategies targeting Tat.

L27 ANSWER 7 OF 69 MEDLINE on STN

2003084225. PubMed ID: 12595763. **Antibody** reactivity to **HIV**-1 Vpu in **HIV**-1/AIDS patients on highly active antiretroviral therapy. Chen Yi-Ming A; Rey Wei-Yu; Lan Yu-Ching; Lai Shu-Fen; Huang Yu-Chuan; Wu Shiow-Ing; Liu Tze-Tze; Hsiao Kwang-Jen. (AIDS Prevention and Research Center, School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC.. arthur@ym.edu.tw) . Journal of biomedical science, (2003 Mar-Apr) 10 (2) 266-75. Journal code: 9421567. ISSN: 1021-7770. Pub. country: Switzerland. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) Vpu protein promotes both extracellular release of viral particles and degradation of CD4 in the endoplasmic reticulum. The correlation of anti-Vpu **antibody** (Ab) reactivity to Vpu and AIDS disease progression was studied in 162 **HIV**-1/AIDS patients after they had received highly active antiretroviral therapy (HAART) for 1 year. Anti-Vpu Ab reactivity was analyzed by Western blot using a recombinant Vpu protein. Results showed that at baseline (prior to initiation of HAART), 31.5% of patients (51/162) had anti-Vpu Ab. The proportion of anti-Vpu Ab in patients with CD4 counts > or =500, 200-500 and <200/mm(3) were 40.6, 34.7 and 14.3%, respectively (chi(2) test, $p < 0.05$). In addition, decreasing levels of anti-Vpu Ab reactivity were significantly correlated with increasing levels of **HIV**-1 viral load. After receiving HAART for 1 year, 7 of 111 anti-Vpu Ab-negative patients (6.3%) seroconverted (- --> + group) and 8 of 51 anti-Vpu Ab-positive (15.7%) patients became negative (+ --> - group). Among 104 anti-Vpu Ab-negative patients, 40 were selected for analysis of the VPU gene. All of them had an intact VPU gene. Patients were further divided into four groups according to their anti-Vpu Ab serostatus and anti-**HIV**-1 Ab was measured. The results showed that only the anti-Vpu Ab seroconverted group (- --> +) had increased serum levels of anti-**HIV**-1 Abs after 1 year of HAART, while the other three groups (+ --> +, - --> - and + --> -) had decreased serum levels of anti-**HIV**-1 Abs after 1 year of HAART ($p < 0.05$). In conclusion, the presence of anti-Vpu Ab is associated with improved prognosis following **HIV**-1 infection, and seroconversion of anti-Vpu Ab in patients on HAART indicates significant recovery of immunity.



L27 ANSWER 8 OF 69 MEDLINE on STN

2002742839. PubMed ID: 12504543. **Antibody** fragments selected by phage display against the nuclear localization signal of the **HIV**-1 **Vpr** protein inhibit nuclear import in permeabilized and intact cultured cells. Krichevsky A; Graessmann A; Nissim A; Piller S C; Zakai N; Loyter A. (Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel. ) Virology, (2003 Jan 5) 305 (1) 77-92. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The **HIV**-1 **Vpr** protein harbors a nuclear localization signal in its N-terminal domain. A peptide bearing this domain and which is designated VprN has been used as a target to screen a phage display single chain Fv (scFv) library. Here we report the isolation of anti-VprN scFv fragments from this library. The purified scFv fragments were able to bind the VprN peptide in an ELISA-based system and to inhibit VprN-mediated nuclear import in permeabilized as well as in intact microinjected cells. Furthermore, the anti-VprN scFv fragments recognized the full-length recombinant **Vpr** protein and inhibited its nuclear import. The same scFv fragments did not inhibit nuclear import mediated by the nuclear localization signal of the SV40 large T-antigen demonstrating a specific effect. The use of the described inhibitory anti-VprN scFv fragments to study nuclear import of viral karyophilic proteins and their therapeutic potential is discussed.

L27 ANSWER 9 OF 69 MEDLINE on STN

2002697252. PubMed ID: 12457980. Recombinant **Vpr** (rVpr) causes augmentation of **HIV**-1 p24 Ag level in U1 cells through its ability to induce the secretion of TNF. Nakamura Taichi; Suzuki Hisako; Okamoto Takashi; Kotani Syuji; Atsuji Yoriko; Tanaka Toshio; Ito Yasuhiko.

(Department of Microbiology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan. ) Virus research, (2002 Dec) 90 (1-2) 263-8. Journal code: 8410979. ISSN: 0168-1702. Pub. country: Netherlands. Language: English.

AB We have found that an **HIV**-1 accessory gene product **Vpr** enhanced **HIV**-1 reproduction in U1 cells chiefly by the induction of TNF, a proinflammatory cytokine, which was also known to be an activator of **HIV**-1 reproduction. We have generated the functional **HIV**-1 accessory gene product **Vpr** in bacterial cells. **Vpr** was generated in an Escherichia coli system (rVpr), purified with **antibodies** (Ab) to the 16 C-terminal amino acids of **Vpr**. The purified rVpr of 15 kDa was examined for its ability to upregulate **HIV**-1 reproduction in U1 cells, which is a reported function of the authentic **Vpr**. rVpr upregulated **HIV**-1 reproduction in U1 cells in a dose-dependent manner and induced the secretion of TNF. The upregulation of **HIV**-1 by rVpr was completely inhibited not only by anti-**Vpr antibodies** but also by anti-TNF **antibody**. These findings suggested that **Vpr** caused an **HIV**-1 reproduction in U1 cells through the induction of TNF.

L27 ANSWER 10 OF 69 MEDLINE on STN

2002498876. PubMed ID: 12359436. **Vpr**- and Nef-dependent induction of RANTES/CCL5 in microglial cells. Si Qiusheng; Kim Mee-Ohk; Zhao Meng-Liang; Landau Nathaniel R; Goldstein Harris; Lee Sunhee. (Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461, USA. ) Virology, (2002 Sep 30) 301 (2) 342-53. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Microglia are pivotal in the pathogenesis of AIDS dementia, as they serve as the major target of **HIV** infection in the CNS. In addition, activation of microglia correlates best with clinical dementia. Although the beta-chemokine RANTES/CCL5 is important in modulating **HIV** infection as well as cellular activation, no information is available regarding how its expression is regulated in microglia by **HIV**-1. Here we report that RANTES/CCL5 expression is induced in microglia by **HIV**-1, but that this requires infection by **HIV**-1. This conclusion was supported by (1) the delayed kinetics coinciding with viral replication; (2) the lack of effect of X4 viruses; (3) inhibition by the reverse transcriptase inhibitor AZT, and (4) the lack of effect of cytokine antagonists or **antibodies**. Interestingly, RANTES/CCL5 production was dependent on the viral accessory protein **Vpr**, in addition to Nef, demonstrating a novel role for **Vpr** in chemokine induction in primary macrophage-type cells. Furthermore, the specific p38 MAP kinase inhibitor SB203580 augmented chemokine expression in microglia, indicating a negative role played by p38. These data suggest unique features of RANTES/CCL5 regulation by **HIV**-1 in human microglial cells.

L27 ANSWER 11 OF 69 MEDLINE on STN

2002092703. PubMed ID: 11751747. **HIV**-1 **viral protein R** compromises cellular immune function in vivo. Ayyavoo Velpandi; Muthumani Karuppiah; Kudchodkar Sagar; Zhang Donghui; Ramanathan P; Dayes Nathanael S; Kim J J; Sin Jeong-Im; Montaner Luis J; Weiner David B. (Department of Infectious Diseases & Microbiology, University of Pittsburgh, PA 15261, USA. ) International immunology, (2002 Jan) 14 (1) 13-22. Journal code: 8916182. ISSN: 0953-8178. Pub. country: England: United Kingdom. Language: English.

AB **HIV**-1 **viral protein R** (**Vpr**) is a virion-associated gene product that profoundly affects T cell proliferation, induces apoptosis and can affect cytokine production in part through interfering with NF-kappa B-mediated transcription from host cells. Collectively, these effects support that **Vpr** could influence immune activation in vivo. However, this effect of **Vpr** has not been explored previously. Here we examined the effect of **Vpr** expression in an in vivo model system on the induction of antigen-specific immune responses using a DNA vaccine model. **Vpr** co-vaccination significantly altered the immune response to co-delivered antigen. Specifically, in the presence of **Vpr**, inflammation was markedly reduced compared to antigen alone. **Vpr** reduced antigen-specific CD8-mediated cytotoxic T lymphocyte activity and suppressed T(h)1 immune responses in vivo as evidenced by lower levels of

towards a T(h)2 response as determined by the IgG2a:IgG1 ratio. The data support that **Vpr** compromises antigen-specific immune responses and ultimately effector cell function, thus confirming a strong selective advantage to the virus at the expense of the host.

L27 ANSWER 12 OF 69 MEDLINE on STN

2002066813. PubMed ID: 11792063. A retroviral DNA vaccine vector. Smith J M; Torres J V. (Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis 95616, USA. ) Viral immunology, (2001) 14 (4) 339-48. Journal code: 8801552. ISSN: 0882-8245. Pub. country: United States. Language: English.

AB A versatile DNA vaccine (pdIV3) was constructed by replacing the integrase, vif, vpx, and **vpr** genes of a pathogenic simian immunodeficiency virus (SIV) molecular clone with a linker containing unique cloning sites. The 5' long terminal repeat (LTR) is truncated and transcription is controlled by a cytomegalovirus (CMV) promoter. The construct expresses Gag and Env in vitro and noninfectious virus particles are produced from transfected cells. The ability of pdIV3 to promote cellular and humoral immune responses, along with the flexibility of the linker design to allow insertion of immunostimulatory genes in future constructs, makes this a useful base vector for immunization against primate lentiviruses. We present the construction of a retroviral plasmid designed to serve as a template for the development of safe and effective vaccines against primate immunodeficiency retroviruses. This vaccine component should facilitate the simultaneous induction of cellular and humoral immune responses that protect primates against infection with SIV and **human immunodeficiency virus** (**HIV**) and the development of acquired immune deficiency syndrome (AIDS). This plasmid could induce the appropriate immune response required to attack both cell-free and cell-associated viruses. The lack of infectivity, the inability to integrate, and the SIV origin make this construct a safe alternative to attenuated vaccines based on **HIV**. In addition, we intend to develop this construct as an immunotherapeutic approach to lower the viremia in AIDS patients.

L27 ANSWER 13 OF 69 MEDLINE on STN

2001636314. PubMed ID: 11690549. Virion-targeted viral inactivation: new therapy against viral infection. Okui N; Kitamura Y; Kobayashi N; Sakuma R; Ishikawa T; Kitamura T. (Department of Urology, University of Tokyo, Tokyo, Japan.. e-urology@geocities.co.jp) . Molecular urology, (2001 Summer) 5 (2) 59-66. Journal code: 9709255. ISSN: 1091-5362. Pub. country: United States. Language: English.

AB BACKGROUND: Acquired immune deficiency syndrome (AIDS) is resistant to all current therapy. Gene therapy is an attractive alternative or additive to current, unsatisfactory AIDS therapy. MATERIALS AND METHODS: To develop an antiviral molecule targeting viral integrase (**HIV** IN), we generated a single-chain **antibody**, termed scAb, which interacted with **human immunodeficiency virus** type 1 (**HIV**-1) IN and inhibited virus replication at the integration step when expressed intracellularly. To reduce infectivity from within the virus particles, we made expression plasmids (pC-scAbE-**Vpr**, pC-scAbE-CA, and pC-scAbE-WXXF), which expressed the anti-**HIV** IN scAb fused to the N-terminus of **HIV**-1-associated accessory protein R (**Vpr**), capsid protein (CA), and specific binding motif to **Vpr** (WXXF), respectively. All fusion proteins were tagged with a nine-amino acid peptide derived from influenza virus hemagglutinin (HA) at the C terminus. RESULTS: The fusion molecules, termed scAbE-**Vpr**, scAbE-CA, and scAbE-WXXF, interacted specifically with **HIV** IN immobilized on a nitrocellulose membrane. Immunoblot analysis showed that scAbE-**Vpr**, scAbE-CA, and scAbE-WXXF were incorporated into the virions produced by cotransfection of 293T cells with **HIV**-1 infectious clone DNA (pLAI) and pC-scAbE-**Vpr**, pC-scAbE-WXXF. A multinuclear activation galactosidase indicator (MAGI) assay revealed that the virions released from 293T cells cotransfected with pLAI and pC-scAbE-**Vpr**, pC-scAbE-WXXF had as little 1000-fold of the infectivity of the control wild-type virions, which were produced from the 293T cells transfected with pLAI

clone. Furthermore, the virions produced from the 293T cells cotransfected with pLAI and an scAb expression vector (pC-scAb) showed only 1% of the infectivity of the control **HIV**-1 in a MAGI assay, although scAb was not incorporated into the virions. In either instance, the total quantity of the progeny virions released from the transfected 293T cells and the patterns of the virion proteins were hardly affected by the presence of scAb, scAbE-**Vpr**, or scAbE-WXXF, as determined by virion-associated reverse transcriptase assay and by immunoblot analysis, respectively. Because G418-selected HeLa clones carrying the expression plasmid for scAbE-WXXF were obtained much more frequently than those for scAbE-**Vpr**, scAbE-WXXF was inferred to be less toxic to cells than scAbE-**Vpr**. The result that scAbE-WXXF with viral incorporation achieved more than a 10-fold reduction in infectivity of the progeny virions than scAb without incorporation suggests that scAbE-WXXF is a potential antiviral molecule, inhibiting replication by neutralization of **HIV** IN activity both within cells and within virions. Moreover, it is nontoxic to human cells. We termed this gene therapy "virion-"targeted-viral inactivation" and these molecules "packageable antiviral therapeutics." CONCLUSION: This new gene therapy has the potential for wide application in many viral infectious diseases.

L27 ANSWER 14 OF 69 MEDLINE on STN

2001491410. PubMed ID: 11531415. Adaptation to blockade of **human immunodeficiency virus** type 1 entry imposed by the anti-CCR5 monoclonal **antibody** 2D7. Aarons E J; Beddows S; Willingham T; Wu L; Koup R A. (Department of Medicine, Division of Infectious Disease, University of Texas Southwestern Medical Center, Texas, Dallas 75390, USA. ) Virology, (2001 Sep 1) 287 (2) 382-90. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The second extracellular loop (ECL2) domain of CC-chemokine receptor 5 (CCR5) has been proposed as a specific target site for therapeutic agents aimed at blocking CCR5-dependent entry by **human immunodeficiency virus** type I (**HIV**-1). We have adapted two CCR5-using **HIV**-1 isolates, prototypic JR-CSF, and a primary isolate, 11-121, to replicate in vitro in the presence of high concentrations of a monoclonal **antibody** (MAb 2D7) specific for the CCR5 ECL2 domain. The 75% inhibitory concentrations (IC(75)) for the two 2D7-adapted isolates were approximately 100-fold higher than those for corresponding control isolates passaged without the MAb. Adapted isolates did not acquire the ability to use CXCR4, CCR3, or CCR1. Env clones derived from MAb 2D7-adapted JR-CSF showed several gp120 mutations that were not found in any of the control JR-CSF clones. The in vitro observations suggest that CCR5-using **HIV**-1 strains might also be able to adapt in vivo to evade an ECL2-blocking therapeutic agent.


L27 ANSWER 15 OF 69 MEDLINE on STN

2001237958. PubMed ID: 11297684. A complex **human immunodeficiency virus** type 1 A/G/J recombinant virus isolated from a seronegative patient with AIDS from Benin, West Africa. Baldrich-Rubio E; Anagonou S; Stirrups K; Lafia E; Candotti D; Lee H; Allain J P. (Department of Haematology, University of Cambridge, East Anglia Blood Centre, Long Road, Cambridge CB2 2PT, UK. ) Journal of general virology, (2001 May) 82 (Pt 5) 1095-106. Journal code: 0077340. ISSN: 0022-1317. Pub. country: England: United Kingdom. Language: English.

AB A **human immunodeficiency virus** type 1 (**HIV**-1(B76)) originating from Benin (West Africa) was isolated and characterized. The patient had severe clinical AIDS and presented an unusual serological profile. Only one out of five different detection assays was able to demonstrate the presence of **antibodies** to **HIV**, whereas confirmatory assays remained indeterminate. In contrast, both plasma viral load and p24 antigen level were unusually high. **HIV**-1 infection was proved by viral RNA and proviral DNA amplification. **HIV**-1(B76) partially purified lysate reacted strongly with all anti-**HIV**-1-positive sera from the region but B76 plasma did not react with subtype A control viral antigen. This patient is likely to have had severe acquired immune dysfunction

the genome identified a complex **HIV**-1 A/G/J recombinant. The gag and pol genes, and the majority of nef,are characteristic of subtype A; the gag/pol junction, the 3' end of pol, vpu and env genes were characteristic of subtype G; vif, **vpr** and the 5' end of nef were subtype J. In addition, part of the **HIV**-1(B76) genome had considerable sequence similarity with the previously described CRF06 cpx (BFP90) isolate. **HIV**-1(B76) did not exhibit any remarkable replication properties or cell tropism in vitro.

L27 ANSWER 16 OF 69 MEDLINE on STN

2001183372. PubMed ID: 11244567. Choice of expression vector alters the localization of a human cellular protein. Ramanathan M P; Ayyavoo V; Weiner D B. (Department of Pathology & Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA. ) DNA and cell biology, (2001 Feb) 20 (2) 101-5. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

AB The fusion of synthetic epitopes with proteins of interest is an important tool in the identification and characterization of recombinant proteins. Several mammalian expression vectors are commercially available containing unique identification tags or epitopes. These vectors offer a great advantage to researchers, as highly specific **antibodies** and purification resins against these specific epitopes are readily available. The tags facilitate immunologic assays and the purification of the recombinant proteins. The fusion of these epitopes with the recombinant proteins is not expected to alter the behavior of the protein of interest. In this report, we demonstrate that the mere expression of a cellular protein, hVIP/mov34, which we earlier identified as a cellular **HIV**-1 **Vpr** ligand, in two different vectors clearly altered its localization pattern in HeLa cells. Specifically, cloning of hVIP/mov34 in pcDNA3/HisA resulted in its nuclear localization, whereas the expression of this gene from a TOPO cloning expression vector, pcDNA3.1/V5/His, resulted in cytoplasmic expression. The native staining pattern of hVIP/mov34 using polyclonal **antisera** raised against hVIP/mov34 demonstrated cytoplasmic staining. During cloning, other leader sequences intended for targeting this protein into a cytoplasmic or a nuclear location were not fused to the actual ORF of this protein. Also, the amino acid sequence of the fusion region arising from cloning of hVIP/mov34 in both vectors does not match any reported NLS sequences. These results indicate that the choice of the expression vectors, as well as the position of synthetic epitopes, can significantly alter the behavior and the biology of recombinant proteins. This result suggests the need for a careful examination of these features when characterizing a newly identified protein.

L27 ANSWER 17 OF 69 MEDLINE on STN

2000482216. PubMed ID: 10967282. Regulation of cell cycle and apoptosis by **human immunodeficiency virus** type 1 **Vpr**. Fukumori T; Akari H; Yoshida A; Fujita M; Koyama A H; Kagawa S; Adachi A. (Department of Virology, The University of Tokushima School of Medicine, 770-8503, Tokushima, Japan. ) Microbes and infection / Institut Pasteur, (2000 Jul) 2 (9) 1011-7. Journal code: 100883508. ISSN: 1286-4579. Pub. country: France. Language: English.

AB Biological effects of **HIV**-1 **Vpr** on CD4(+) cells were studied by an infection system. High-titered **HIV**-1 stocks pseudotyped with vesicular stomatitis virus G protein were prepared and used to inoculate into CD4(+)T cells at high multiplicity of infection. Both cell- and virion-associated **Vpr** were demonstrated to arrest the cell cycle at the G2/M phase, and to induce cell apoptosis. Of note, morphologically apoptotic cells were shown to be arrested at the G2/M stage. No appreciable effect of **Vpr** on the anti-Fas **antibody**-mediated apoptosis was observed in this system.

L27 ANSWER 18 OF 69 MEDLINE on STN

2000454545. PubMed ID: 10957723. Generation of monoclonal **antibodies** specifically directed against the proximal zinc finger of **HIV** type 1 NCp7. De Rocquigny H; Caneparo A; Dong C Z; Delaunay T; Roques B P.

(Département de Pharmacochimie Moléculaire et Structurale, INSERM U266-CNRS UMR 8600, UFR des Sciences Pharmaceutiques et Biologiques, Paris, France. ) AIDS research and human retroviruses, (2000 Sep 1) 16 (13) 1259-67. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB The **HIV**-1 NCp7 contains two spatially close zinc fingers, required for the production of infectious particles. To investigate in more detail the function of the zinc finger domain, monoclonal **antibodies** were generated with a cyclic analog of the NCp7 proximal zinc finger. This analog was shown to bind zinc ions and to preserve the highly folded structure of the native peptide (Dong C-Z et al.: J Am Chem Soc 1995;117:2726-2731). We report here two monoclonal **antibodies** (2B10 and 4D3), which are the first monoclonal **antibodies** directed against CCHC NCp7 zinc fingers. Dot-blot experiments revealed that a few nanograms of synthetic NCp7 can be detected on a nitrocellulose membrane. Whereas 2B10 appears specific for an epitope located in sequence 19-27 of NCp7, 4D3 appears to be structurally specific. Immunocomplex affinities were evaluated, using BIAcore technology, to be up to 1 and 10 nM, respectively, for 2B10 and 4D3 in 100 mM NaCl. These **antibodies** were able to recognize NCp7 in the Gag polyprotein precursor and were shown to immunoprecipitate NCp7 from a cell supernatant. Moreover, NCp7-**Vpr** interaction mediated by the zinc fingers is inhibited by 2B10, emphasizing the role of these domains in the protein-protein complex. These results indicate that 2B10 and 4D3 behave as useful tools for studying both NC protein functions during the course of virion morphogenesis and the role played by its zinc finger domain at various steps in the retroviral life cycle.

L27 ANSWER 19 OF 69 MEDLINE on STN

2000431184. PubMed ID: 10877854. Antiviral agent based on the non-structural protein targeting the maturation process of **HIV**-1: expression and susceptibility of chimeric **Vpr** as a substrate for cleavage by **HIV**-1 protease. Serio D; Singh S P; Cartas M A; Weber I T; Harrison R W; Louis J M; Srinivasan A. (Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA. ) Protein engineering, (2000 Jun) 13 (6) 431-6. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The processing of precursor proteins (Gag and Gag-pol) by the viral protease is absolutely required in order to generate infectious particles. This prompted us to consider novel strategies that target viral maturation. Towards this end, we have engineered an **HIV**-1 virion associated protein, **Vpr**, to contain protease cleavage signal sequences from Gag and Gag-pol precursor proteins. We previously reported that virus particles derived from **HIV**-1 proviral DNA, encoding chimeric **Vpr**, showed a lack of infectivity, depending on the fusion partner. As an extension of that work, the potential of chimeric **Vpr** as a substrate for **HIV**-1 protease was tested utilizing an epitope-based assay. Chimeric **Vpr** molecules were modified such that the Flag epitope is removed following cleavage, thus allowing us to determine the efficiency of protease cleavage. Following incubation with the protease, the resultant products were analyzed by radioimmunoprecipitation using **antibodies** directed against the Flag epitope. Densitometric analysis of the autoradiograms showed processing to be both rapid and specific. Further, the analysis of virus particles containing chimeric **Vpr** by immunoblot showed reactivities to **antibodies** against the Flag epitope similar to the data observed in vitro. These results suggest that the pseudosubstrate approach may provide another avenue for developing antiviral agents.

L27 ANSWER 20 OF 69 MEDLINE on STN

2000387923. PubMed ID: 10888660. Two putative alpha-helical domains of **human immunodeficiency virus** type 1 **Vpr** mediate nuclear localization by at least two mechanisms. Kamata M; Aida Y. (RIKEN Tsukuba Institute, Tsukuba, Ibaraki 305-0074, Japan. ) Journal of virology, (2000 Aug) 74 (15) 7179-86. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB To identify the domains of **Vpr** that are involved nuclear localization, we transfected HeLa cells with a panel of expression vectors that encode mutant **Vpr** protein with deletions or substitutions within putative domains. Immunofluorescence staining of transfected cells revealed that wild-type **Vpr** was localized predominantly in the nucleus and the nuclear envelope and certainly in the cytoplasm. Introduction of substitutions or deletions within alphaH1 or alphaH2 resulted, by contrast, in diffuse expression over the entire cell. In addition, double mutations within both of these alpha-helical domains led to the complete absence of **Vpr** from nuclei. Next, we prepared HeLa cells that express chimeric proteins which consist of the alphaH1 and alphaH2 domains fused individually with green fluorescent protein (GFP) and a Flag tag and extracted them with digitonin and Triton X-100 prior to fixation. Flag-alphaH1-GFP was detected in the nucleus but not in the cytoplasm, while Flag-alphaH2-GFP was retained predominantly in the nucleus and in a small amount in the cytoplasm. The immunostaining patterns were almost eliminated by substitutions in each chimeric protein. Thus, it appeared that the two alpha-helical domains might be involved in nuclear import by binding to certain cellular factors. Taken together, our data suggest that the two putative alpha-helical domains mediate the nuclear localization of **Vpr** by at least two mechanisms.

L27 ANSWER 21 OF 69 MEDLINE on STN

2000235040. PubMed ID: 10819575. Gene transfer into stimulated and unstimulated T lymphocytes by **HIV**-1-derived lentiviral vectors. Costello E; Munoz M; Buetti E; Meylan P R; Diggelmann H; Thali M. (Institute of Microbiology, University of Lausanne, Switzerland. ) Gene therapy, (2000 Apr) 7 (7) 596-604. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Genetic modification of T lymphocytes holds great potential for treatments of cancer, T cell disorders and AIDS. While in the past recombinant murine retroviruses were the vectors of choice for gene delivery to T cells, vectors based on lentiviruses can provide additional benefits. Here, we show that VSV-G pseudotyped **HIV** 1 vector particles delivering the enhanced green fluorescent protein (EGFP) efficiently transduce human T lymphocytes. Transduction efficiency was optimal when infection included centrifugation of cells with concentrated vector supernatant in the presence of Polybrene. In contrast to previous reports describing murine retrovirus-mediated gene transfer to T lymphocytes, fibronectin did not improve the transduction efficiency of the VSVG-pseudotyped **HIV**-1 particles. Similar gene transfer efficiencies were observed following stimulation of cells with PHA/IL-2 or anti-CD3i/CD28i **antibodies**, although greater transgene expression was observed in the latter case. Interestingly, production of vectors in the absence of the accessory proteins Vif, **Vpr**, Vpu and Nef was accompanied by a 50% decrease in transduction efficiency in activated T cells. Transduction of T cells that were not stimulated before infection was achieved. No transduction of non-prestimulated cells was observed with a GAL V-pseudotyped murine retroviral vector. The requirement for accessory proteins in non-prestimulated cells was more pronounced. Our results have implications for lentiviral vector targeting of other cells of the hematopoietic system including stem cells.

L27 ANSWER 22 OF 69 MEDLINE on STN

2000234835. PubMed ID: 10774802. Inhibition of **HIV**-1 replication and infectivity by expression of a fusion protein, **VPR**-anti-integrase single-chain variable fragment (SFv): intravirion molecular therapies. BouHamdan M; Kulkosky J; Duan L X; Pomerantz R J. (Center for Human Virology, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA. ) Journal of human virology, (2000 Jan-Feb) 3 (1) 6-15. Journal code: 9805755. ISSN: 1090-9508. Pub. country: United States. Language: English.

AB OBJECTIVES: To deliver antiretroviral agents or other foreign proteins into progeny virions and evaluate their inhibitory effect on **human immunodeficiency virus** type 1 (**HIV**-1) replication. STUDY DESIGN/METHODS: **HIV**-1 encodes proteins in addition to gag, pol, and env,

some of which are packaged into virus particles. One essential retroviral enzyme is integrase (IN), which has been used as a target for developing agents that inhibit virus replication. In previous studies, we demonstrated that intracellular expression of single-chain variable **antibody** fragments (SFvs), which bind to IN, results in resistance to productive **HIV**-1 infection in T-lymphocytic cells. Because the highly conserved accessory **HIV**-1 **Vpr** protein can be packaged within virions in quantities similar to those of the major structural proteins, this primate lentiviral protein may be used as a fusion partner to deliver antiviral agents or other foreign proteins into progeny virions. In these studies, the fusion proteins **Vpr**-chloramphenicol acetyl transferase (CAT) and **Vpr**-SFv-IN have been developed. Stable transfectants expressing these fusion proteins were generated from PA317 cells and SupT1 T-lymphocytic cells and analyzed using immunofluorescence microscopy. After challenge of SupT1 cells with **HIV**-1, p24 antigen expression was evaluated. The incorporation of these fusion proteins were evaluated by immunoprecipitation of virions using a **Vpr antibody**. RESULTS: Expression of the fusion proteins was confirmed by immunofluorescent staining in PA317 cells transfected with the plasmids expressing **Vpr**-CAT and **Vpr**-SFv-IN proteins. Stable transfectants expressing these fusion proteins were generated from SupT1 T-lymphocytic cells. When challenged, **HIV**-1 replication, as measured by **HIV**-1 p24 antigen expression, was inhibited in cells expressing **Vpr**-SFv-IN. It was demonstrated that **Vpr**-chloramphenicol acetyl transferase (**Vpr**-CAT and **Vpr**-SFv-IN proteins can be efficiently packaged into the virions and that **Vpr**-SFv-IN also decreases the infectivity of virions into which it is encapsidated. CONCLUSIONS: An anti-integrase single-chain variable fragment moiety can be delivered into **HIV**-1 virions by fusing it to **Vpr**. **Vpr**-SFv-IN decreases **HIV**-1 production in human T-lymphocytic cells. The benefits of "intravirion" gene therapy include immunization of target cells as well as decreasing infectivity of **HIV**-1 virions harboring the fusion construct. Thus, this approach to anti-**HIV**-1 molecular therapies has the potential to increase inhibitory effects against **HIV**-1 replication and virion spread.

L27 ANSWER 23 OF 69 MEDLINE on STN

2000186599. PubMed ID: 10724032. Packageable antiviral therapeutics against **human immunodeficiency virus** type 1: virion-targeted virus inactivation by incorporation of a single-chain **antibody** against viral integrase into progeny virions. Okui N; Sakuma R; Kobayashi N; Yoshikura H; Kitamura T; Chiba J; Kitamura Y. (Division of Molecular Genetics, National Institute of Infectious Diseases, Tokyo, Japan. ) Human gene therapy, (2000 Mar 1) 11 (4) 537-46. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB To determine their activities as an antiviral agent packageable within virions and suitable for continued expression in cells, we tested a single-chain **antibody** (scAb) against **human immunodeficiency virus** type 1 (**HIV**-1) integrase and its three fusion proteins: fused to **viral protein R** (scab-**Vpr**), a double-cassette of the WXXF motif binding to **Vpr** (scAb-WXXF), and viral major capsid protein (scAb-CA), respectively. Cotransfection of human 293T cells with expression plasmid for scAb-**Vpr** or -WXXF along with **HIV**-1 clone pLAI resulted in the production of a normal amount of progeny virions with infectivity decreased by more than 10(3)-fold. Immunoblot analyses showed that scAb-**Vpr** or -WXXF was associated with virions, whereas scAb or scAb-CA was not, suggesting that scAb-**Vpr** or -WXXF was incorporated into virions. The incorporation of scAb-WXXF appeared to be **Vpr** dependent, because the fusion protein was associated with the wild-type but not with **Vpr**-truncated **HIV**-1 virions. Since G418-selected HeLa clones carrying expression plasmid for scAb-WXXF were obtained much more frequently than those for scAb-**Vpr**, scAb-WXXF was inferred to be less toxic to cells than scAb-**Vpr**. These results suggest that scAb-WXXF may serve as a novel class of antiviral therapeutic that inactivates progeny **HIV** virions from within.

L27 ANSWER 24 OF 69 MEDLINE on STN

2000179645. PubMed ID: 10713269. Extent of incorporation of **HIV**-1 **Vpr**

into the virus particles is flexible and can be modulated by expression level in cells. Lai D; Singh S P; Cartas M; Murali R; Kalyanaraman V S; Srinivasan A. (Department of Microbiology and Immunology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA 19107, USA. ) FEBS letters, (2000 Mar 10) 469 (2-3) 191-5. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB To examine the factors that control the extent of incorporation of **Vpr** into the virus particles, we utilized an epitope-tagging approach with Flag (FL) as the epitope for quantitation. We generated expression plasmids containing **Vpr**-FL and **Vpr** E21,24P-FL and also **HIV**-1 proviral DNA containing **Vpr**-FL (NL-**Vpr**-FL). Immunoblot analysis using Flag **antibodies** revealed that virus particles derived from co-transfection of NL-**Vpr**-FL and **Vpr**-FL showed an enhanced level of **Vpr**-FL in comparison to NL-**Vpr**-FL derived virus. These results suggest that the amount of incorporation of **Vpr** into the virus particles is flexible and may be modulated by its expression level in cells.

L27 ANSWER 25 OF 69 MEDLINE on STN

2000111559. PubMed ID: 10646090. New prospects for the development of a vaccine against **human immunodeficiency virus** type 1. An overview. Girard M; Habel A; Chanel C. (Departement de virologie, Institut Pasteur, Paris, France.. mgirard@pasteur.fr) . Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie, (1999 Nov) 322 (11) 959-66. Ref: 69. Journal code: 8503078. ISSN: 0764-4469. Pub. country: France. Language: English.

AB During the past few years, definite progress has been made in the field of **human immunodeficiency virus** type 1 (**HIV**-1) vaccines. Initial attempts using envelope gp120 or gp140 from T-cell line-adapted (TCLA) **HIV**-1 strains to vaccinate chimpanzees showed that neutralizing **antibody**-based immune responses were protective against challenge with homologous TCLA virus strains or strains with low replicative capacity, but these neutralizing **antibodies** remained inactive when tested on primary **HIV**-1 isolates, casting doubts on the efficacy of gp120-based vaccines in the natural setting. Development of a live attenuated simian immunodeficiency virus (SIV) vaccine was undertaken in the macaque model using whole live SIV bearing multiple deletions in the nef, **vpr** and vpx genes. This vaccine provided remarkable protective efficacy against wild-type SIV challenge, but the deletion mutants remain pathogenic, notably in neonate monkeys. Study of the mechanisms of protection in the SIV model unravelled the importance of the T-cell responses, whether in the form of cytotoxic T-lymphocyte (CTL) killing activity, or in that of antiviral factor secretion of cytokines, beta-chemokines and other unidentified antiviral factors by CD8+ T-cells. Induction of such a response is being sought at this time using various live recombinant virus vaccines, either poxvirus or alphavirus vectors or DNA vectors, which can be combined together or with a gp120/gp140 boost in various prime-boost combination strategies. New vectors include attenuated vaccinia virus NYVAC, modified vaccinia strain Ankara (MVA), Semliki Forest virus, Venezuelan equine encephalitis virus, and Salmonellas. Recent DNA prime-poxvirus boost combination regimens have generated promising protection results against SIV or SIV/**HIV** (SHIV) challenge in macaque models. Emphasis is also put on the induction of a mucosal immune response, involving both a secretory IgA response and a mucosal CTL response which could constitute a 'first line of defence' in the vaccinated host. Finally, a totally novel vaccine approach based on the use of Tat or Tat and Rev antigens has been shown to induce efficient protection from challenge with pathogenic SIV or SHIV in vaccinated macaques. The only vaccine in phase 3 clinical trials in human volunteers is a gp120-based vaccine, AIDSVAX. A prime-boost combination of a recombinant canarypoxvirus and a subunit gp120 vaccine is in phase 2. Emphasis has been put recently on the necessity of testing prototype vaccines in developing countries using immunogens derived from local virus strains. Trial sites have thus been identified in Kenya, Uganda, Thailand and South Africa where phase I trials have begun or are expected to start presently.

2000070315. PubMed ID: 10600597. Protection of macaques against a SHIV with a homologous **HIV**-1 Env and a pathogenic SHIV-89.6P with a heterologous Env by vaccination with multiple gene-deleted SHIVs. Ui M; Kuwata T; Igarashi T; Ibuki K; Miyazaki Y; Kozyrev I L; Enose Y; Shimada T; Uesaka H; Yamamoto H; Miura T; Hayami M. (Institute for Virus Research, Kyoto University, Kyoto, 606-8507, Japan. ) Virology, (1999 Dec 20) 265 (2) 252-63. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB To evaluate the potential of SHIVs as anti-**HIV**-1 live vaccines, we constructed two gene-deleted SHIVs, designated SHIV-drn and SHIV-dxrn. The former lacks **vpr**/nef and the latter lacks vpx/**vpr**/nef. Four macaques that had been vaccinated with SHIV-drn were challenged with SHIV-NM-3rN, which has an **HIV**-1 Env that is the same as that of SHIV-drn. No challenge virus was detected by DNA PCR in, or recovered from, two of the macaques. In the other two, challenge virus was detected once and twice, respectively. Plasma viral loads were much lower than those in unvaccinated controls. Another four macaques were vaccinated with SHIV-dxrn. These macaques showed resistance but less than that of SHIV-drn-vaccinated macaques. When the two SHIV-drn-vaccinated macaques were challenged with pathogenic SHIV-89.6P, which has an **HIV**-1 Env that is antigenically different from that of SHIV-drn, replication of the challenge virus was restricted, and the usual decrease in the number of CD4(+) cells was prevented. In this protection, it is noteworthy that protection involved not only neutralizing **antibodies** and killer cell activity, but also other unknown specific and nonspecific immunity elicited by the infection.


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2000059064. PubMed ID: 10593491. Protective immunity of gene-deleted SHIVs having an **HIV**-1 Env against challenge infection with a gene-intact SHIV. Ui M; Kuwata T; Igarashi T; Miyazaki Y; Tamaru K; Shimada T; Nakamura M; Uesaka H; Yamamoto H; Hayami M. (Laboratory of Viral Pathogenesis, Institute for Virus Research, Kyoto, Japan. ) Journal of medical primatology, (1999 Aug-Oct) 28 (4-5) 242-8. Journal code: 0320626. ISSN: 0047-2565. Pub. country: Denmark. Language: English.

AB We constructed three simian-human immunodeficiency viruses (SHIVs) lacking regulatory gene(s) and analyzed their induction of protective immunity against challenge infection with gene-intact SHIV in rhesus macaques. Inoculation of SHIV-dn lacking nef and SHIV-drn lacking nef and **vpr** induced transient viremia, while that of SHIV-dxrn lacking nef, **vpr**, and vpx induced no viremia. The SHIVs with fewer deletions were more effective in inducing neutralizing **antibodies** and cytotoxic T lymphocyte responses. When these macaques were challenged with parental gene-intact SHIV-NM-3rN, all the SHIV-dn-vaccinated macaques and two of the four SHIV-drn-vaccinated macaques showed complete resistance. The other two SHIV-drn-vaccinated macaques and all SHIV-dxrn-vaccinated macaques did not show complete resistance, but they did show suppression of replication of the challenge virus. These results suggested that as more genes were deleted, protective immunity was decreased.

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2000040674. PubMed ID: 10570196. Emergence of a highly pathogenic simian/**human immunodeficiency virus** in a rhesus macaque treated with anti-CD8 mAb during a primary infection with a nonpathogenic virus. Igarashi T; Endo Y; Englund G; Sadjadpour R; Matano T; Buckler C; Buckler-White A; Plishka R; Theodore T; Shibata R; Martin M. (Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. ) Proceedings of the National Academy of Sciences of the United States of America, (1999 Nov 23) 96 (24) 14049-54. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Although simian/**human immunodeficiency virus** (SHIV) strain DH12 replicates to high titers and causes immunodeficiency in pig-tailed macaques, virus loads measured in SHIV(DH12)-infected rhesus monkeys are

developed disease. We previously reported that the administration of anti-human CD8 mAb to rhesus macaques at the time of primary SHIV(DH12) infection resulted in marked elevations of virus loads. One of the treated animals experienced rapid and profound depletions of circulating CD4(+) T lymphocytes. Although the CD4(+) T cell number partially recovered, this monkey subsequently suffered significant weight loss and was euthanized. A tissue culture virus stock derived from this animal, designated SHIV(DH12R), induced marked and rapid CD4(+) cell loss after i.v. inoculation of rhesus monkeys. Retrospective analyses of clinical specimens, collected during the emergence of SHIV(DH12R) indicated: (i) the input cloned SHIV remained the predominant virus during the first 5-7 months of infection; (ii) variants bearing only a few of the SHIV(DH12R) consensus changes first appeared 7 months after the administration of anti-CD8 mAb; (iii) high titers of neutralizing **antibody** directed against the input SHIV were detected by week 10 and persisted throughout the infection; and (iv) no neutralizing **antibody** against SHIV(DH12R) ever developed.

L27 ANSWER 29 OF 69 MEDLINE on STN

1999370180. PubMed ID: 10438826. Characterization of a highly replicative intergroup M/O **human immunodeficiency virus** type 1 recombinant isolated from a Cameroonian patient. Peeters M; Liegeois F; Torimiro N; Bourgeois A; Mpoudi E; Vergne L; Saman E; Delaporte E; Saragosti S. (Institut de Recherche pour le Developpement, Montpellier, France.. martine.peeters@mpl.ird.fr) . Journal of virology, (1999 Sep) 73 (9) 7368-75. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A Cameroonian patient with **antibodies** reacting simultaneously to **human immunodeficiency virus** type 1 (**HIV**-1) group O- and group M-specific V3-loop peptides was identified. In order to confirm that this patient was coinfected with both viruses, PCRs with O- and M-specific discriminating primers corresponding to different regions of the genome were carried out with both primary lymphocyte DNA and the corresponding viral strains isolated from three consecutive patient samples. The PCR data suggested that this patient is coinfected with a group M virus and a recombinant M/O virus. Indeed, only type M gag sequences could be amplified, while for the env region, both type M and O sequences were amplified, from plasma or from DNA extracted from primary lymphocytes. Sequence analysis of a complete recombinant genome isolated from the second sample (97CA-MP645 virus isolate) revealed two intergroup breakpoints, one in the **vpr** gene and the second in the long terminal repeat region around the TATA box. Comparison of the type M sequences shared by the group M and the recombinant M/O viruses showed that these sequences were closely related, with only 3% genetic distance, suggesting that the M virus was one of the parental viruses. In this report we describe for the first time a recombination event in vivo between viruses belonging to two different groups, leading to a replicative virus. Recombination between strains with such distant lineages (65% overall homology) may contribute substantially to the emergence of new **HIV**-1 variants. We documented that this virus replicates well and became predominant in vitro. At this time, group O viruses represent a minority of the strains responsible for the **HIV**-1 pandemic. If such recombinant intergroup viruses gained better fitness, inducing changes in their biological properties compared to the parental group O virus, the prevalences of group O sequences could increase rapidly. This will have important implications for diagnosis of **HIV**-1 infections by serological and molecular tests, as well as for antiviral treatment.

L27 ANSWER 30 OF 69 MEDLINE on STN

1999262160. PubMed ID: 10329448. Direct visualization of **HIV**-1 entry: mechanisms and role of cell surface receptors. Stauber R H; Rulong S; Palm G; Tarasova N I. (ABL-Basic Research Program, NCI-FCRDC, Frederick, Maryland, 21702-1201, USA. ) Biochemical and biophysical research communications, (1999 May 19) 258 (3) 695-702. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Highly fluorescent virions of T- and M-tropic **HIV**-1 isolates were obtained by incorporation of the viral accessory protein **Vpr**, fused to the green fluorescent protein, in trans. The fluorescent virions displayed normal morphology, were infectious, and could be used for direct visualization of **HIV**-1 attachment and trafficking in various cell lines. More than 90% of the viral particles were found to enter the cells by direct membrane fusion in T-cells, CD4+ HeLa cells, and macrophages. Visualizing **HIV**-1 attachment and entry in the absence or presence of CD4 and/or the appropriate coreceptors indicated that CD4 is the major receptor for virus attachment in the case of JR-CSF and NL-4-3 **HIV**-1 isolates; however, the coreceptors are required for membrane fusion. Internalization of the coreceptor CXCR4 inhibited entry, but did not prevent virus binding suggesting that transient downregulation of the coreceptor(s) may not be the most efficient way of blocking **HIV** infection in vivo.


L27 ANSWER 31 OF 69 MEDLINE on STN
1999262107. PubMed ID: 10329395. Multiple centrosome formation induced by the expression of **Vpr** gene of **human immunodeficiency virus**.
Minemoto Y; Shimura M; Ishizaka Y; Masamune Y; Yamashita K. (Graduate School of Natural Science and Technology, Kanazawa University, 13-1, Kanazawa, Takara-machi, 920-0934, Japan. ) Biochemical and biophysical research communications, (1999 May 10) 258 (2) 379-84. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB We previously established a cell line called MIT-23 in which expression of the **Vpr** gene of **human immunodeficiency virus** 1 (**HIV**-1) can be controlled by the addition of tetracycline. **Vpr** expression induces multiple nuclear formation and increased ploidy in MIT-23 cells. We herein report that multipolar mitotic spindles were formed upon induction of **Vpr**. Further analysis of centrosomes with anti-gamma-tubulin immunostaining revealed that a significant population of cells 1 week after expression of **Vpr** gene product had an increased number of centrosomes in the cells with abnormal nuclei. Taking into account that the centrosome plays an important role in genome integrity, the abnormal number of centrosomes in cells expressing **Vpr** may be directly related to aneuploidy or the formation of micronuclei in MIT-23 cells, suggesting that **Vpr** has an oncogenic role in **HIV** infected cells.


L27 ANSWER 32 OF 69 MEDLINE on STN
1999253892. PubMed ID: 10321975. Replication of different clones of **human immunodeficiency virus** type 1 in primary fetal human astrocytes: enhancement of viral gene expression by Nef. Bencheikh M; Bentsman G; Sarkissian N; Canki M; Volsky D J. (Molecular Virology Laboratory, St. Luke's/Roosevelt Hospital Center, Columbia University, New York, USA. ) Journal of neurovirology, (1999 Apr) 5 (2) 115-24. Journal code: 9508123. ISSN: 1355-0284. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Dementia is a common complication of AIDS which is associated with **human immunodeficiency virus** type 1 (**HIV**-1) infection of brain macrophages and microglia. Recent studies have shown that astrocytes are also infected in the brain but **HIV**-1 replication in these cells is restricted. To determine virus specificity of this restriction we tested the expression of 15 **HIV**-1 molecular clones in primary human fetal astrocytes by infection and DNA transfection. Infection with cell-free viruses was poorly productive and revealed no clone-specific differences. In contrast, transfected cells produced transiently high levels of **HIV**-1 p24 core antigen, up to 50 nanograms per ml culture supernatant, and nanogram levels of p24 were detected 3-4 weeks after transfection of some viral clones. The average peak expression of **HIV**-1 in astrocytes varied as a function of viral clone used by a factor of 15 but the differences and the subsequent virus spread did not correlate with the tropism of the viral clones to T cells or macrophages. Functional vif, vpu, and **vpr** genes were dispensable for virus replication from transfected DNA, but intact nef provided a detectable enhancement of early viral gene

that primary astrocytes present no fundamental barriers to moderate expression of different strains of **HIV**-1 and that the presence of functional Nef is advantageous to virus infection in these cells.

L27 ANSWER 33 OF 69 MEDLINE on STN

1999247324. PubMed ID: 10232364. Gene-mutated **HIV**-1/SIV chimeric viruses as AIDS live attenuated vaccines for potential human use. Hayami M; Igarashi T; Kuwata T; Ui M; Haga T; Ami Y; Shinohara K; Honda M. (Institute for Virus Research, Kyoto University, Japan. ) Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K, (1999 Apr) 13 Suppl 1 S42-7. Journal code: 8704895. ISSN: 0887-6924. Pub. country: ENGLAND: United Kingdom. Language: English.

AB To develop an AIDS vaccine for human use as well as a suitable animal model for AIDS research, we constructed a series of **HIV**-1/SIVmac chimeric viruses (SHIVs). We successfully generated a SHIV (designated as NM-3rN) having the **HIV**-1 env gene, which enabled the evaluation of the efficacy of **HIV**-1 Env-targeted vaccines in macaque monkeys instead of chimpanzees. Two NM-3rN derivatives (NM-3 and NM-3n) induced long-term anti-virus immunities without manifesting the disease. The monkeys vaccinated with NM-3 or NM-3n became resistant to a challenge inoculation with NM-3rN. Serum from a monkey vaccinated with NM-3 neutralized not only the parental **HIV**-1 (NL432), but also an antigenically different **HIV**-1 (MN). In vivo experiments confirmed the heterologous protection against an SHIV having the **HIV**-1 (MN) env. In addition to specific immunity including neutralizing **antibodies** and cytotoxic T lymphocyte activity, nonspecific immunity such as natural killer activity is associated with this protection. These data suggest that the live vaccine has the ability to protect individuals against various types of HIVs. These SHIVs should contribute to the development of future anti-**HIV**-1 live vaccines in humans.

L27 ANSWER 34 OF 69 MEDLINE on STN

1998325226. PubMed ID: 9658154. Production of uninfectious **human immunodeficiency virus** type 1 containing **viral protein R** fused to a single-chain **antibody** against viral integrase. Okui N; Kobayashi N; Kitamura Y. (Division of Molecular Genetics, National Institute of Infectious Diseases, Gakuen, Musashimurayama, Tokyo, Japan. ) Journal of virology, (1998 Aug) 72 (8) 6960-4. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A single-chain **antibody** (scAb) against **human immunodeficiency virus** type 1 (**HIV**-1) integrase was expressed as a fusion protein of scAb and **HIV**-1 **viral protein R** (**Vpr**), together with the **HIV**-1 genome, in human 293T cells. The expression did not affect virion production much but markedly reduced the infectivity of progeny virions. The fusion protein was found to be incorporated into the virions. The incorporation appears to account for the reduced infectivity.

L27 ANSWER 35 OF 69 MEDLINE on STN

1998278831. PubMed ID: 9611258. Direct binding to nucleic acids by **Vpr** of **human immunodeficiency virus** type 1. Zhang S; Pointer D; Singer G; Feng Y; Park K; Zhao L J. (Institute for Molecular Virology, St. Louis University School of Medicine, 3681 Park Avenue, St. Louis, MO 63110, USA. ) Gene, (1998 Jun 8) 212 (2) 157-66. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB **Human immunodeficiency virus** type 1 (**HIV**-1) **viral protein R** (**Vpr**) is a 15kDa regulatory protein packaged in the **HIV**-1 virion. Although the molecular mechanism of **Vpr** function during viral replication remains elusive, **Vpr** has been found to possess interesting biological activities, including cell-cycle arrest at the G2/M check point, promotion of the **HIV**-1 pre-integration complex for nuclear transport, and a low but significant level of transcriptional activation of a variety of viral and cellular promoters. We now present data suggesting that **HIV**-1 **Vpr** is a nucleic-acid-binding protein. This activity of **Vpr** was demonstrated by DNA-cellulose chromatography, **antibody** co-immunoprecipitation, and gel electrophoretic mobility shift

assays. By mutational analysis, the C-terminal region of **Vpr**, which is rich in basic amino-acid residues, was shown to be critical for **Vpr** binding to nucleic acids. The nucleic-acid-binding activity of **Vpr** is consistent with several biological activities of **Vpr** and may provide an important clue for understanding the molecular interactions between **HIV**-1 and the host cells.

L27 ANSWER 36 OF 69 MEDLINE on STN

1998250796. PubMed ID: 9582382. **Viral protein R** regulates docking of the **HIV**-1 preintegration complex to the nuclear pore complex. Popov S; Rexach M; Ratner L; Blobel G; Bukrinsky M. (The Picower Institute for Medical Research, Manhasset, New York 11030, USA. ) Journal of biological chemistry, (1998 May 22) 273 (21) 13347-52. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Replication of **human immunodeficiency virus** type 1 (**HIV**-1) in non-dividing cells depends critically on import of the viral preintegration complex into the nucleus. Recent evidence suggests that **viral protein R** (**Vpr**) plays a key regulatory role in this process by binding to karyopherin alpha, a cellular receptor for nuclear localization signals, and increasing its affinity for the nuclear localization signals. An in vitro binding assay was used to investigate the role of **Vpr** in docking of the **HIV**-1 preintegration complex (PIC) to the nuclear pore complex. Mutant **HIV**-1 PICs that lack **Vpr** were impaired in the ability to dock to isolated nuclei and recombinant nucleoporins. Although **Vpr** by itself associated with nucleoporins, the docking of **Vpr**+ PICs was dependent on karyopherin beta and was blocked by **antibodies** to beta. **Vpr** stabilized docking by preventing nucleoporin-stimulated dissociation of the import complex. These results suggest a biochemical mechanism for **Vpr** function in transport of the **HIV**-1 genome across the nuclear pore complex.

L27 ANSWER 37 OF 69 MEDLINE on STN

1998190361. PubMed ID: 9514978. Arginine residues in the C-terminus of **HIV**-1 **Vpr** are important for nuclear localization and cell cycle arrest. Zhou Y; Lu Y; Ratner L. (Division of Molecular Oncology, Washington University School of Medicine, St. Louis, Missouri 63110, USA. ) Virology, (1998 Mar 15) 242 (2) 414-24. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB **HIV**-1 **viral protein R** (**Vpr**) is predominantly localized to the nucleus and plays an important role for viral preintegration complex import into the nucleus. In this study, we investigated the influence on subcellular localization of Arg residues in the C-terminus of **Vpr**. Consistent with previous studies, about 90% of the cells manifested diffuse nuclear staining in the **Vpr**-expressed cells. Besides diffuse nuclear staining, punctate perinuclear staining, and punctate cytoplasmic staining were also observed in the immunofluorescence studies. Deletion of the Ser-Arg-Ile-Gly residues (amino acids 79-82; SRIG) had no effect on the **Vpr** localization. However, deletion of the Arg-Gln-Arg-Arg residues (amino acids 85-88; RQRR) resulted in a smooth perinuclear staining pattern. Substitution of five Arg residues with Asn (amino acids 80, 85, 87, 88, and 90; R-->N5) resulted in a diffuse cytoplasmic staining. Subcellular fractionation analyses support the immunofluorescence staining results. These findings indicate that the C-terminal Arg residues of **HIV**-1 **Vpr** play an important role for **Vpr** nuclear localization. All the **Vpr** mutants were appropriately expressed, exhibited no significant defect on the protein stability, and were incorporated efficiently into virus-like particles. Both SRIG and R-->N5 mutants lost their cell cycle arrest activities and the RQRR deletion only exhibited a low level of cell arrest activity. Therefore, the Arg residues in the **HIV**-1 **Vpr** C-terminus are important for **Vpr** nuclear localization and cell cycle arrest, but had no effect on protein stability or **Vpr** incorporation into virus-like particles.

L27 ANSWER 38 OF 69 MEDLINE on STN

1998184698. PubMed ID: 9525784. Infectivity and immunogenicity of SIVmac/**HIV**-1 chimeric viruses (SHIVs) with deletions in two or three

genes (**vpr**, nef and vpu). Igarashi T; Kanuka H; Tsunetsugu-Yokota Y; Nishiyama K; Ui M; Miyazaki Y; Hayami M. (Laboratory of Pathogenic Virus, Institute for Virus Research, Kyoto University, Japan. ) Microbiology and immunology, (1998) 42 (1) 71-4. Journal code: 7703966. ISSN: 0385-5600. Pub. country: Japan. Language: English.

AB Two SHIVs with two or three genes deleted (SHIV-drn and SHIV-dxrn) were constructed. The inoculation of monkeys with SHIV-drn resulted in short-term viremia, but inoculation with SHIV-dxrn did not. At 68 weeks post-inoculation, the monkeys were reinoculated with a 100-fold higher dose of each SHIV, but none showed viremia. Killer cell activities against **HIV**-1 Env were detected in the SHIV-drn- and SHIV-dxrn-inoculated monkeys. Cross-reactive killer activity against **HIV**-1 Gag and SIVmac Gag was observed in one monkey. **Antibodies** were not detected in the SHIV-dxrn-inoculated monkeys, but the SHIV-drn-inoculated monkeys showed an anamnestic **antibody** reaction. These data indicate that SHIV-drn is infectious to and immuno-inducible in macaques but SHIV-dxrn is not.

L27 ANSWER 39 OF 69 MEDLINE on STN

1998119890. PubMed ID: 9449720. The **HIV**-1 **vpr** protein acts as a negative regulator of apoptosis in a human lymphoblastoid T cell line: possible implications for the pathogenesis of AIDS. Conti L; Rainaldi G; Matarrese P; Varano B; Rivabene R; Columba S; Sato A; Belardelli F; Malorni W; Gessani S. (Laboratory of Virology, Istituto Superiore di Sanita, Viale Regina Elena, 299-00161 Rome, Italy. ) Journal of experimental medicine, (1998 Feb 2) 187 (3) 403-13. Journal code: 2985109R. ISSN: 0022-1007. Pub. country: United States. Language: English.

AB Although apoptosis is considered one of the major mechanisms of CD4(+) T cell depletion in **HIV**-infected patients, the virus-infected cells somehow appear to be protected from apoptosis, which generally occurs in bystander cells. **Vpr** is an auxiliary **HIV**-1 protein, which, unlike the other regulatory gene products, is present at high copy number in virus particles. We established stable transfectants of CD4+ T Jurkat cells constitutively expressing low levels of **vpr**. These clones exhibited cell cycle characteristics similar to those of control-transfected cells. Treatment of control clones with apoptotic stimuli (i.e., cycloheximide/tumor necrosis factor alpha (TNF-alpha), anti-Fas **antibody**, or serum starvation) resulted in a massive cell death by apoptosis. In contrast, all the **vpr**-expressing clones showed an impressive protection from apoptosis independently of the inducer. Notably, **vpr** antisense phosphorothioate oligodeoxynucleotides render **vpr**-expressing cells as susceptible to apoptosis induced by cycloheximide and TNF-alpha as the control clones. Moreover, the constitutive expression of **HIV**-1 **vpr** resulted in the upregulation of bcl-2, an oncogene endowed with antiapoptotic activities, and in the downmodulation of bax, a proapoptotic factor of the bcl-2 family. Altogether, these results suggest that low levels of the endogenous **vpr** protein can interfere with the physiological turnover of T lymphocytes at early stages of virus infection, thus facilitating **HIV** persistence and, subsequently, viral spread. This might explain why apoptosis mostly occurs in bystander uninfected cells in AIDS patients.

L27 ANSWER 40 OF 69 MEDLINE on STN

1998099744. PubMed ID: 9436978. **HIV**-1 **Vpr** interacts with the nuclear transport pathway to promote macrophage infection. Vodicka M A; Koepp D M; Silver P A; Emerman M. (Divisions of Molecular Medicine and Basic Science, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109 USA. ) Genes & development, (1998 Jan 15) 12 (2) 175-85. Journal code: 8711660. ISSN: 0890-9369. Pub. country: United States. Language: English.

AB **HIV**-1 **Vpr** promotes nuclear entry of viral nucleic acids in nondividing macrophages and also causes a G2 cell-cycle arrest. Consistent with its role in nuclear transport, we show **Vpr** localizes to the nuclear envelope in both human and yeast cells. Like the importin-beta subunit of the nuclear import receptor, **Vpr** also interacts with the yeast importin-alpha subunit and nucleoporins. Moreover, overexpression of either **Vpr** or importin-beta in yeast blocks nuclear transport of mRNAs.

A mutant form of **Vpr** (**Vpr** 1-81), that does not localize at the nuclear envelope, or bind to importin-alpha and nucleoporins, renders **HIV**-1 incapable of infecting macrophages efficiently. **Vpr** F34I, however, still causes a G2 arrest, demonstrating that the dual functions of **Vpr** are genetically separable. Our data suggest **Vpr** functionally resembles importin-beta in nuclear import of the **HIV**-1 pre-integration complex and this function is essential for the role of **Vpr** in macrophage infection, but not G2 arrest.

L27 ANSWER 41 OF 69 MEDLINE on STN

97296945. PubMed ID: 9152414. Protection of monkeys vaccinated with **vpr**-and/or nef-defective simian immunodeficiency virus strain mac/**human immunodeficiency virus** type 1 chimeric viruses: a potential candidate live-attenuated human AIDS vaccine. Igarashi T; Ami Y; Yamamoto H; Shibata R; Kuwata T; Mukai R; Shinohara K; Komatsu T; Adachi A; Hayami M. (Laboratory of Pathogenic Virus, Kyoto University, Japan. ) Journal of general virology, (1997 May) 78 ( Pt 5) 985-9. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Two simian immunodeficiency virus strain mac (SIVmac)/**human immunodeficiency virus** type 1(**HIV**-1) chimeric viruses (SHIVs), designated NM-3 and NM-3n, with env derived from **HIV**-1 and defective **vpr** (plus defective nef for NM-3), were inoculated into seven macaques. These macaques were transiently or persistently infected and most of them produced long-lasting neutralizing **antibodies** and Env-specific killer T cells to **HIV**-1 with no AIDS-like symptoms. When they were challenged with another SHIV with intact **vpr** and nef (designated NM-3rN), all were protected as judged by virus recovery, DNA detection by PCR and **antibody** responses. Anti-**HIV**-1 Env-specific killer T cells were considered to have played a major role in this protection, but a non-specific defence mechanism as well as specific immunity also appeared to be involved. Thus, these two non-pathogenic SHIVs induced long-lasting protective immunities in macaques, suggesting the possibility of gene-defective SHIVs as attenuated live vaccines for human use.

L27 ANSWER 42 OF 69 MEDLINE on STN

97057722. PubMed ID: 8902061. Chimeric viruses between SIVmac and various **HIV**-1 isolates have biological properties that are similar to those of the parental **HIV**-1. Kuwata T; Shioda T; Igarashi T; Ido E; Ibuki K; Enose Y; Stahl-Hennig C; Hunsmann G; Miura T; Hayami M. (Institute for Virus Research, Kyoto University, Japan. ) AIDS (London, England), (1996 Oct) 10 (12) 1331-7. Journal code: 8710219. ISSN: 0269-9370. Pub. country: ENGLAND: United Kingdom. Language: English.

AB OBJECTIVE: To examine the biological properties of **HIV**-1/SIVmac chimeric viruses from **HIV**-1 isolates that have different replication rates, cell tropisms and cytopathicities. DESIGN AND METHODS: Four chimeric viruses with gag, pol, vif, vpx, nef and long terminal repeats of SIVmax and **vpr**, tat, rev, vpu and env of various **HIV**-1 isolates were constructed and compared in vitro. Cynomolgus monkeys were inoculated with two chimeras that were replicative in monkey peripheral blood mononuclear cells (PBMC). RESULTS: The type-specific neutralization of the chimeras by monoclonal **antibodies** 0.5 beta and mu 5.5, which recognize V3 of **HIV**-1IIIB and **HIV**-1MN respectively, was observed to be similar to those of the parental viruses, **HIV**-1NL432, **HIV**-1HAN2 and **HIV**-1SF13. The chimeras constructed from **HIV**-1SF2 and **HIV**-1SF13, which were isolates from the same individual but from different disease stages, reflected their parental properties, that is, the isolate from the later stage was rapid-high replicating, was more cytopathic and had a wider host range. Chimeras constructed from **HIV**-1HAN2' **HIV**-1SF13 and **HIV**-1NL432 were infectious to macaque monkeys, although the monkeys infected with the chimera from **HIV**-1SF13 showed lower virus loads and shorter viremic periods than those infected with the others. CONCLUSIONS: Chimeras have in vitro properties that are similar to those of their parental **HIV**-1 isolates, but their growth in macaque PBMC was dependent on which **HIV**-1 isolate was used. Evaluation of a vaccine by challenging with viruses possessing different antigenicities has become possible in macaque monkeys using newly constructed chimeras.

L27 ANSWER 43 OF 69 MEDLINE on STN

96413214. PubMed ID: 8816376. Apoptosis induced by **HIV** infection in H9 T cells is blocked by ICE-family protease inhibition but not by a Fas(CD95) antagonist. Glynn J M; McElligott D L; Mosier D E. (Department of Immunology-IMM-7, Scripps Research Institute, La Jolla, CA 92037, USA. ) Journal of immunology (Baltimore, Md. : 1950), (1996 Oct 1) 157 (7) 2754-8. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Infection of human CD4-positive T lymphocytes by **human immunodeficiency virus** type 1 (**HIV**-1) is thought to lead to death of infected cells by apoptosis, although one recent report questions this conclusion. Here we demonstrate that **HIV**-1-induced apoptosis of the H9 human T cell line is blocked by peptide inhibitors of IL-1 beta converting enzyme (ICE)-family proteases, but not by the antagonistic M3 anti-Fas Ab. Apoptosis occurred in all phases of the cell cycle, not selectively in G2 as a consequence of **vpr**-mediated cell cycle arrest. We conclude that apoptosis accounts for all cell death related to **HIV**-1 infection of the human CD4-positive cell line H9, requires an ICE-like protease but is not Fas mediated, and occurs in all phases of the cell cycle.

L27 ANSWER 44 OF 69 MEDLINE on STN

96133887. PubMed ID: 8552585. **Vpr** protein of **human immunodeficiency virus** type 1 forms cation-selective channels in planar lipid bilayers. Piller S C; Ewart G D; Premkumar A; Cox G B; Gage P W. (John Curtin School of Medical Research, Australian National University, Canberra, Australia. ) Proceedings of the National Academy of Sciences of the United States of America, (1996 Jan 9) 93 (1) 111-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A small (96-aa) protein, virus protein R (**Vpr**), of **human immunodeficiency virus** type 1 contains one hydrophobic segment that could form a membrane-spanning helix. Recombinant **Vpr**, expressed in Escherichia coli and purified by affinity chromatography, formed ion channels in planar lipid bilayers when it was added to the cis chamber and when the trans chamber was held at a negative potential. The channels were more permeable to Na+ than to Cl- ions and were inhibited when the trans potential was made positive. Similar channel activity was caused by **Vpr** that had a truncated C terminus, but the potential dependence of channel activity was no longer seen. **Antibody** raised to a peptide mimicking part of the C terminus of **Vpr** (AbC) inhibited channel activity when added to the trans chamber but had no effect when added to the cis chamber. **Antibody** to the N terminus of **Vpr** (AbN) increased channel activity when added to the cis chamber but had no effect when added to the trans chamber. The effects of potential and **antibodies** on channel activity are consistent with a model in which the positive C-terminal end of dipolar **Vpr** is induced to traverse the bilayer membrane when the opposite (trans) side of the membrane is at a negative potential. The C terminus of **Vpr** would then be available for interaction with AbC in the trans chamber, and the N terminus would be available for interaction with AbN in the cis chamber. The ability of **Vpr** to form ion channels in vitro suggests that channel formation by **Vpr** in vivo is possible and may be important in the life cycle of **human immunodeficiency virus** type 1 and/or may cause changes in cells that contribute to AIDS-related pathologies.

L27 ANSWER 45 OF 69 MEDLINE on STN

95306144. PubMed ID: 7786585. Polyclonal rabbit **antisera** that detect the **Vpr** protein of SIVSM and SIVMAC on immunoblots of purified virions. Newman M A; McPherson S A; Fletcher T M 3rd; Kappes J C; Hahn B H. (Department of Medicine, University of Alabama at Birmingham 35294, USA. ) AIDS research and human retroviruses, (1995 Mar) 11 (3) 405-8. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB **Antisera** suitable for detection of SIVSM or SIVMAC **Vpr** proteins on Western blots of purified virions are currently not available. We have expressed the **Vpr** protein of SIVSMPBj1.9 in a gst-based prokaryotic

expression system and used it to raise polyclonal **antisera** in rabbits. Two immune sera were obtained that specifically recognized both cell- and virion-associated **Vpr** protein on immunoblots of three different SIV isolates (SIVSMPBj1.9, SIVMACBK28, and SIVMAC239). Because **Vpr** is believed to play an important role in **HIV**/SIV replication and pathogenesis, these reagents will allow the extension of functional analyses of this protein to a broader spectrum of viruses. Both **antisera** and the gst-**Vpr** expression plasmid have been submitted to the NIAID AIDS Research and Reagent Program and are available to interested investigators.

L27 ANSWER 46 OF 69 MEDLINE on STN

95306142. PubMed ID: 7786583. Humoral and cellular immune responses in rhesus macaques infected with **human immunodeficiency virus** type 2. Abimiku A G; Franchini G; Aldrich K; Myagkikh M; Markham P; Gard E; Gallo R C; Robert-Guroff M. (Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA. ) AIDS research and human retroviruses, (1995 Mar) 11 (3) 383-93. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Eighteen rhesus macaques were inoculated with either an infectious molecularly cloned **human immunodeficiency virus** type 2 (**HIV**-2)SBL/ISY, or with one of eight mutants defective in one or more accessory genes. The immune responses generated by the macaques were monitored for up to 2 years postinfection. All the macaques except those that received mutants lacking the **vpr** or vif genes demonstrated low to moderate **antibody** titers. Macaques inoculated with vpx- mutants exhibited a persistent serological response, suggesting continuous virus expression even in the absence of detectable virus in the peripheral blood mononuclear cells (PBMCs). Neutralizing **antibodies** developed in only four macaques. In general, low-level cytotoxic T lymphocyte (CTL) activity, not clearly **HIV**-2 specific, was detected in PBMCs. However, one virus-negative macaque exhibited significant **HIV**-2-specific CTL activity in an enriched CD8+ cell population from PBMCs, suggesting clearance of the viral infection. In addition, CTL activity against the Env and Gag/Pol epitopes of **HIV**-2 by CD8+ lymphocytes from the spleens and lymph nodes of two infected macaques, in one case requiring CD8+ T cell enrichment and in the other clearly evident in unfractionated tissue lymphocytes, was demonstrated for the first time. This sequestration of tissue CTLs occurred in the absence of significant levels of circulating CTLs in the blood. Our results suggest that routine monitoring of PBMCs may sometimes be inadequate for detecting cell-mediated immune responses. Elucidation of immune correlates of vaccine protection may therefore require sampling of lymphoid tissues and assessment of enriched CD8+ populations.

L27 ANSWER 47 OF 69 MEDLINE on STN

95297167. PubMed ID: 7778297. Fusogenic determinants of highly cytopathic subtype D Zairian isolate **HIV**-1 NDK. De Mareuil J; Salaun D; Chermann J C; Hirsch I. (Unite de Recherches sur les Retrovirus et Maladies Associees, INSERM U322, Parc Scientifique et Technologique de Luminy, Marseille, France. ) Virology, (1995 Jun 1) 209 (2) 649-53. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Phenotypic characterization of subtype B strains of **human immunodeficiency virus** type 1 (**HIV**-1) indicates that the major determinants of their cytopathogenicity and tropism are contained in the gene coding for the envelope glycoprotein gp120, namely in its variable regions V1, V2, and V3. Recombinant viruses derived from **HIV**-1 LAV, the subtype B prototype virus, and **HIV**-1 NDK, the Zairian subtype D virus highly cytopathic for CD4-positive lymphocytes, were used to elucidate genetic control of fusogenic functions in subtype D viruses. Our data demonstrate that multigenic determination of fusogenic properties is more complex in the subtype D than in clade B viruses. Variability in three regions of **HIV**-1 NDK genome correlated with formation of large syncytia. These regions consisted of the matrix protein, the C-terminal portion of **vpr** up to the C1 region of gp120, and the V1-V3 regions of gp120.

variability in the envelope glycoprotein but not in other regions of the **HIV**-1 genome was related to enhanced resistance of **HIV**-1 NDK to treatment of target cells with OKT4-A anti-CD4 MAb. Therefore, a different genetic control affects two aspects of **HIV**-1 fusogenicity: (i) variability in the envelope glycoprotein itself is sufficient to influence a virus-to-cell fusion at the virus/cell entry, and (ii) a more complex genetic function including genes of matrix protein and envelope glycoprotein is related to variability of cell-to-cell fusion during formation of syncytium.

L27 ANSWER 48 OF 69 MEDLINE on STN

95264427. PubMed ID: 7745685. Targeting foreign proteins to **human immunodeficiency virus** particles via fusion with **Vpr** and Vpx. Wu X; Liu H; Xiao H; Kim J; Seshaiah P; Natsoulis G; Boeke J D; Hahn B H; Kappes J C. (Department of Medicine, University of Alabama at Birmingham 35294. ) Journal of virology, (1995 Jun) 69 (6) 3389-98. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus** type 1 (**HIV**-1) and **HIV**-2 **Vpr** and Vpx proteins are packaged into virions through virus type-specific interactions with the Gag polyprotein precursor. To examine whether **HIV**-1 **Vpr** (Vpr1) and **HIV**-2 Vpx (Vpx2) could be used to target foreign proteins to the **HIV** particle, their open reading frames were fused in frame with genes encoding the bacterial staphylococcal nuclease (SN), an enzymatically inactive mutant of SN (SN*), and chloramphenicol acetyltransferase (CAT). Transient expression in a T7-based vaccinia virus system demonstrated the synthesis of appropriately sized Vpr1-SN/SN* and Vpx2-SN/SN* fusion proteins which, when coexpressed with their cognate p55Gag protein, were efficiently incorporated into virus-like particles. Packaging of the fusion proteins was dependent on virus type-specific determinants, as previously seen with wild-type **Vpr** and Vpx proteins. Particle-associated Vpr1-SN and Vpx2-SN fusion proteins were enzymatically active, as determined by in vitro digestion of lambda phage DNA. To determine whether functional Vpr1 and Vpx2 fusion proteins could be targeted to **HIV** particles, the gene fusions were cloned into an **HIV**-2 long terminal repeat/Rev response element-regulated expression vector and cotransfected with wild-type **HIV**-1 and **HIV**-2 proviruses. Western blot (immunoblot) analysis of sucrose gradient-purified virions revealed that both Vpr1 and Vpx2 fusion proteins were efficiently packaged regardless of whether SN, SN*, or CAT was used as the C-terminal fusion partner. Moreover, the fusion proteins remained enzymatically active and were packaged in the presence of wild-type **Vpr** and Vpx proteins. Interestingly, virions also contained smaller proteins that reacted with **antibodies** specific for the accessory proteins as well as SN and CAT fusion partners. Since similar proteins were absent from Gag-derived virus-like particles and from virions propagated in the presence of an **HIV** protease inhibitor, they must represent cleavage products produced by the viral protease. Taken together, these results demonstrate that **Vpr** and Vpx can be used to target functional proteins, including potentially deleterious enzymes, to the human or simian immunodeficiency virus particle. These properties may be exploitable for studies of **HIV** particle assembly and maturation and for the development of novel antiviral strategies.

L27 ANSWER 49 OF 69 MEDLINE on STN

95191013. PubMed ID: 7884883. Progression to AIDS in the absence of a gene for **vpr** or vpx. Gibbs J S; Lackner A A; Lang S M; Simon M A; Sehgal P K; Daniel M D; Desrosiers R C. (New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772-9102. ) Journal of virology, (1995 Apr) 69 (4) 2378-83. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Rhesus monkeys (Macaca mulatta) were experimentally infected with strains of simian immunodeficiency virus (SIV) derived from SIVmac239 lacking **vpr**, vpx, or both **vpr** and vpx genes. These auxiliary genes are not required for virus replication in cultured cells but are consistently conserved within the SIVmac/**human immunodeficiency virus** type 2/SIVsm group of primate lentiviruses. All four rhesus monkeys infected

with the **vpr** deletion mutant showed an early spike in plasma antigenemia, maintained high virus burdens, exhibited declines in CD4+ lymphocyte concentrations, and had significant changes in lymph node morphology, and two have died to date with AIDS. The behavior of the **vpr** deletion mutant was indistinguishable from that of the parental, wild-type virus. Rhesus monkeys infected with the vpx deletion mutant showed lower levels of plasma antigenemia, lower virus burdens, and delayed declines in CD4+ lymphocyte concentrations but nonetheless progressed with AIDS to a terminal stage. The **vpr**+vpx double mutant was severely attenuated, with much lower virus burdens and no evidence of disease progression. These and other results indicate that **vpr** provides only a slight facilitating advantage for wild-type SIVmac replication in vivo. Thus, progression to AIDS and death can occur in the absence of a gene for **vpr** or vpx.

L27 ANSWER 50 OF 69 MEDLINE on STN

95115082. PubMed ID: 7815499. Extracellular **Vpr** protein increases cellular permissiveness to **human immunodeficiency virus** replication and reactivates virus from latency. Levy D N; Refaeli Y; Weiner D B. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104-4283. ) Journal of virology, (1995 Feb) 69 (2) 1243-52. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The **vpr** gene product of **human immunodeficiency virus** (**HIV**) and simian immunodeficiency virus is a virion-associated regulatory protein that has been shown using **vpr** mutant viruses to increase virus replication, particularly in monocytes/macrophages. We have previously shown that **vpr** can directly inhibit cell proliferation and induce cell differentiation, events linked to the control of **HIV** replication, and also that the replication of a **vpr** mutant but not that of wild-type **HIV** type 1 (**HIV**-1) was compatible with cellular proliferation (D. N. Levy, L. S. Fernandes, W. V. Williams, and D. B. Weiner, Cell 72:541-550, 1993). Here we show that purified recombinant **Vpr** protein, in concentrations of < 100 pg/ml to 100 ng/ml, increases wild-type **HIV**-1 replication in newly infected transformed cell lines via a long-lasting increase in cellular permissiveness to **HIV** replication. The activity of extracellular **Vpr** protein could be completely inhibited by anti-**Vpr antibodies**. Extracellular **Vpr** also induced efficient **HIV**-1 replication in newly infected resting peripheral blood mononuclear cells. Extracellular **Vpr** transcomplemented a **vpr** mutant virus which was deficient in replication in promonocytic cells, restoring full replication competence. In addition, extracellular **Vpr** reactivated **HIV**-1 expression in five latently infected cell lines of T-cell, B-cell, and promonocytic origin which normally express very low levels of **HIV** RNA and protein, indicating an activation of translational or pretranslational events in the virus life cycle. Together, these results describe a novel pathway governing **HIV** replication and a potential target for the development of anti-**HIV** therapeutics.

L27 ANSWER 51 OF 69 MEDLINE on STN

95062167. PubMed ID: 7971975. Serum **Vpr** regulates productive infection and latency of **human immunodeficiency virus** type 1. Levy D N; Refaeli Y; MacGregor R R; Weiner D B. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia 19104. ) Proceedings of the National Academy of Sciences of the United States of America, (1994 Nov 8) 91 (23) 10873-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB In **human immunodeficiency virus** (**HIV**)-positive individuals, the vast majority of infected peripheral blood cells and lymph node cells may be latently or nonproductively infected. The **vpr** open reading frame of **HIV**-1 encodes a 15-kDa virion-associated protein, **Vpr**. The **vpr** gene has been shown to increase virus replication in T cells and monocyte/macrophages in vitro. We have previously reported that **vpr** expression in various tumor lines leads to growth inhibition and differentiation, indicating that **Vpr** may function as a regulator of cellular permissiveness to **HIV** replication. Here we show that **Vpr**

protein is present in significant amounts in the serum of HIV patients. Purified serum **Vpr** activated virus expression from five latently infected cell lines, U1, OM.10.1, ACH-2, J1.1, and LL58. Serum **Vpr** also activated virus expression from resting peripheral blood mononuclear cells of **HIV**-infected individuals. Together, these findings implicate serum **Vpr** in the activation of **HIV** replication in vivo and in the control of latency. Anti-**Vpr antibodies** inhibited **Vpr** activity, suggesting that humoral immunity modulates **Vpr** activity in vivo. These results have broad implications for the virus life cycle and for the prospective control of **HIV** replication and pathogenesis.

L27 ANSWER 52 OF 69 MEDLINE on STN

94267704. PubMed ID: 8207641. Induction of neutralizing **antibodies** against **human immunodeficiency virus** type 1 using synthetic peptide constructs containing an immunodominant T-helper cell determinant from **vpr**. Sarobe P; Lasarte J J; Golvano J J; Prieto I; Gullon A; Soto M J; Labarga P; Prieto J; Borras-Cuesta F. (Departamento de Medicina Interna, Universidad de Navarra, Pamplona, Spain. ) Journal of acquired immune deficiency syndromes, (1994 Jul) 7 (7) 635-40. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB Identification of immunodominant T-helper-cell determinants after natural infection is an important step in the design of immunogens for potential use in vaccination. Using cells from **human immunodeficiency virus** type 1 (**HIV**-1)-infected individuals and a panel of peptides encompassing the sequence of the regulatory protein **vpr** from **HIV**-1, we identified the T-helper determinant QLLFIHFRIGCRHSR, which is active in 37.5% of these individuals. To gain insight on the efficacy of this peptide in helping induce neutralizing **antibodies** against a B-cell determinant (BD), we synthesized constructs containing B- and T-cell determinants and tested them in BALB/c mice, the highest responders to the T-cell determinant moiety among several strains tested. These immunogens induced **antibodies** against two chosen B-cell determinants from **HIV**-1IIIB gp160 (amino acids 310-322 from the V3 loop of gp120 and 736-751 from gp41) that were able to neutralize **HIV**-1 infection in vitro. The highest neutralization titer against **HIV**-1IIIB was obtained by immunization with the homopolymer of the construct containing the T-cell epitope from **vpr** and the B-cell epitope from the V3 loop. We believe that the immunodominant T-cell determinant from **vpr** is a promising epitope to consider in the design of future peptide vaccines.

L27 ANSWER 53 OF 69 MEDLINE on STN

94149887. PubMed ID: 8107252. Requirement of the Pr55gag precursor for incorporation of the **Vpr** product into **human immunodeficiency virus** type 1 viral particles. Lavallee C; Yao X J; Ladha A; Gottlinger H; Haseltine W A; Cohen E A. (Departement de Microbiologie et Immunologie, Faculte de Medecine, Universite de Montreal, Quebec, Canada. ) Journal of virology, (1994 Mar) 68 (3) 1926-34. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The **human immunodeficiency virus** type 1 (**HIV**-1) particles consists of two molecules of genomic RNA as well as molecules originating from gag, pol, and env products, all synthesized as precursor proteins. The 96-amino-acid **Vpr** protein, the only virion-associated **HIV**-1 regulatory protein, is not part of the virus polyprotein precursors, and its incorporation into virus particles must occur by way of an interaction with a component normally found in virions. To investigate the mechanism of incorporation of **Vpr** into the **HIV**-1 virion, **Vpr**- proviral DNA constructs harboring mutations or deletions in specific virion-associated gene products were cotransfected with **Vpr** expressor plasmids in COS cells. Virus released from the transfected cells was tested for the presence of **Vpr** by immunoprecipitation with **Vpr**-specific **antibodies**. The results of these experiments show that **Vpr** is trans-incorporated into virions but at a lower efficiency than when **Vpr** is expressed from a proviral construct. The minimal viral genetic information necessary for **Vpr** incorporation was a deleted provirus encoding only the pr55gag polyprotein precursor. Incorporation of **Vpr** requires the expression but not the processing of gag products and is independent of pol and env

expression. Direct incubation of **Vpr** with the Pr55gag precursor protein was demonstrated by coprecipitation experiments with gag product-specific **antibodies**. Overall, these results indicate that **HIV**-1 **Vpr** is incorporated into the nascent virion through an interaction with the Gag precursor polyprotein and demonstrate a novel mechanism by which viral protein can be incorporated into virus particles.

L27 ANSWER 54 OF 69 MEDLINE on STN

94047336. PubMed ID: 8230445. Incorporation of **Vpr** into **human immunodeficiency virus** type 1 virions: requirement for the p6 region of gag and mutational analysis. Paxton W; Connor R I; Landau N R. (Aaron Diamond AIDS Research Center, New York, New York. ) Journal of virology, (1993 Dec) 67 (12) 7229-37. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The product of the **vpr** open reading frame of **human immunodeficiency virus** type 1 (**HIV**-1) is a 15-kDa, arginine-rich protein that is present in virions in molar quantities equivalent to that of Gag. We report here the results of our investigations into the mechanism by which **Vpr** is incorporated into virions during assembly in infected cells. For these studies we used an expression vector encoding a **Vpr** molecule fused at its amino terminus to a nine-amino-acid peptide from influenza virus hemagglutinin. The tagged **Vpr** expression vector and a **vpr** mutant **HIV**-1 provirus were used to cotransfect COS cells, and the resulting virions were tested for the presence of the tagged protein on immunoblots probed with monoclonal **antibody** against the hemagglutinin peptide. The COS-produced virions were found to contain readily detectable amounts of tagged **Vpr** and smaller amounts of a putative tagged **Vpr** dimer. Infectivity of the particles was not altered by incorporation of tagged **Vpr**. Our results using this system in combination with mutant **HIV**-1 proviruses suggested that incorporation of **Vpr** into virions requires the carboxy-terminal Gag protein of **HIV**-1 (p6) but not gp160, Pol, or genomic viral RNA. In addition, analysis of mutated, tagged **Vpr** molecules suggested that amino acids near the carboxy terminus (amino acids 84 to 94) are required for incorporation of **Vpr** into **HIV**-1 virions. The single cysteine residue near the carboxy terminus was required for production of a stable protein. Arginine residues tested were not important for incorporation or stability of tagged **Vpr**. These results suggested a novel strategy for blocking **HIV**-1 replication.

L27 ANSWER 55 OF 69 MEDLINE on STN

94016837. PubMed ID: 8411357. **Human immunodeficiency virus** type 1 **viral protein R** localization in infected cells and virions. Lu Y L; Spearman P; Ratner L. (Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110. ) Journal of virology, (1993 Nov) 67 (11) 6542-50. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The subcellular localization of **human immunodeficiency virus** type 1 (**HIV**-1) **viral protein R** (**Vpr**) was examined by subcellular fractionation. In **HIV**-1-infected peripheral blood mononuclear cells, **Vpr** was found in the nuclear and membrane fractions as well as the conditioned medium. Expression of **Vpr** without other **HIV**-1 proteins, in two different eukaryotic expression systems, demonstrated a predominant localization of **Vpr** in the nuclear matrix and chromatin extract fractions. Deletion of the carboxyl-terminal 19-amino-acid arginine-rich sequence impaired **Vpr** nuclear localization. Indirect immunofluorescence confirmed the nuclear localization of **Vpr** and also indicated a perinuclear location. Expression of **Vpr** alone did not result in export of the protein from the cell, but when coexpressed with the Gag protein, **Vpr** was exported and found in virus-like particles. A truncated Gag protein, missing the p6 sequence and a portion of the p9 sequence, was incapable of exporting **Vpr** from the cell. Regulation of **Vpr** localization may be important in the influence of this protein on virus replication.

L27 ANSWER 56 OF 69 MEDLINE on STN

94016835. PubMed ID: 8411355. Analysis of simian immunodeficiency virus

Sequence variation in tissues of rhesus macaques with simian AIDS: Kodama T; Mori K; Kawahara T; Ringler D J; Desrosiers R C. (Division of Primate Medicine, Oregon Regional Primate Research Center, Medical Research Foundation of Oregon, Beaverton 97006. ) Journal of virology, (1993 Nov) 67 (11) 6522-34. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB One rhesus macaque displayed severe encephalomyelitis and another displayed severe enterocolitis following infection with molecularly cloned simian immunodeficiency virus (SIV) strain SIVmac239. Little or no free anti-SIV **antibody** developed in these two macaques, and they died relatively quickly (4 to 6 months) after infection. Manifestation of the tissue-specific disease in these macaques was associated with the emergence of variants with high replicative capacity for macrophages and primary infection of tissue macrophages. The nature of sequence variation in the central region (vif, **vpr**, and vpx), the env gene, and the nef long terminal repeat (LTR) region in brain, colon, and other tissues was examined to see whether specific genetic changes were associated with SIV replication in brain or gut. Sequence analysis revealed strong conservation of the intergenic central region, nef, and the LTR. However, analysis of env sequences in these two macaques and one other revealed significant, interesting patterns of sequence variation. (i) Changes in env that were found previously to contribute to the replicative ability of SIVmac for macrophages in culture were present in the tissues of these animals. (ii) The greatest variability was located in the regions between V1 and V2 and from "V3" through C3 in gp120, which are different in location from the variable regions observed previously in animals with strong **antibody** responses and long-term persistent infection. (iii) The predominant sequence change of D-->N at position 385 in C3 is most surprising, since this change in both SIV and **human immunodeficiency virus** type 1 has been associated with dramatically diminished affinity for CD4 and replication in vitro. (iv) The nature of sequence changes at some positions (146, 178, 345, 385, and "V3") suggests that viral replication in brain and gut may be facilitated by specific sequence changes in env in addition to those that impart a general ability to replicate well in macrophages. These results demonstrate that complex selective pressures, including immune responses and varying cell and tissue specificity, can influence the nature of sequence changes in env.

L27 ANSWER 57 OF 69 MEDLINE on STN

93224894. PubMed ID: 8468557. In vitro non-productive infection of purified natural killer cells by the BRU isolate of the **human immunodeficiency virus** type 1. Scott-Algara D; Vuillier F; Cayota A; Rame V; Guetard D; Moncany M L; Marasescu M; Dauguet C; Dighiero G. (Unite d'ImmunoHematologie et d'ImmunoPathologie, Institut Pasteur, Paris, France. ) Journal of general virology, (1993 Apr) 74 ( Pt 4) 725-31. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Highly purified natural killer (NK) cell lines and clones, displaying the typical phenotype, morphology and function and obtained from healthy blood donors, were infected in vitro with the BRU isolate of **human immunodeficiency virus** type 1 (**HIV**-1). There was no significant increase in reverse transcriptase activity and levels of p24 antigen in the supernatants, but positive staining was observed using an immunogold technique with polyclonal anti-**HIV**-1 **antibodies**. When infected NK cells were co-cultivated with autologous non-infected CD4+ mitogen-activated cells, significant levels of reverse transcriptase activity and p24 antigen in supernatants were detected. Giant syncytial cells and a high number of mature virion particles were also evident. When NK cell lines or clones from **HIV**-1-infected patients were studied, neither the presence of p24 antigen nor reverse transcriptase activity was detected in the supernatants after stimulation with mitogens, cytokines or co-culture with allogeneic CD4+ mitogen-activated cells. PCR studies did not detect **HIV**-1 genes in freshly purified NK cells, cell lines or clones from infected patients. Taken together these results suggest that (i) normal NK cells can be infected in vitro by the **HIV**-1 BRU isolate in a non-productive fashion, (ii) PCR with NK cell DNA of **HIV**-1-infected

patients indicates that in vivo few of these cells, if any, are infected by **HIV**-1 and (iii) the mechanisms responsible for the impairment of NK cell function during **HIV**-1 infection remain to be determined and are probably not related to a direct cytopathic effect of the virus.

L27 ANSWER 58 OF 69 MEDLINE on STN

93110974. PubMed ID: 1281948. Development of a vaccine for the prevention of AIDS, a critical appraisal. Karzon D T; Bolognesi D P; Koff W C. (Department of Pediatrics, Vanderbilt Medical School, Nashville, TN 37232. ) Vaccine, (1992) 10 (14) 1039-52. Ref: 108. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The pathogenesis and clinical expression of **HIV**-1 infection in humans is considered in terms of classical pathogenetic studies of viral infections for which successful vaccines have been produced. The unique features of **HIV** pathogenesis are defined, and gaps in knowledge identified as a framework for considering designs for immune intervention. Envelope-derived candidate vaccines have been used in immunization and challenge experiments in SIV/macaque or **HIV**/chimpanzee models, presented either as vaccinia recombinant vectors or as subunits, singly or in sequence. These studies have been paralleled by clinical trials for safety and immunogenicity in seronegative individuals. Data generated will permit comparison of immune responses to specific antigens and delivery systems in animal models and in humans. In limited studies conducted under optimized conditions, non-human primates have been protected against virus challenge when immunized with some candidate vaccines or following passive transfer of high-titred **antibody**. Consideration of current information suggests that in order to prevent **HIV** infection it may be necessary to devise new strategies capable of inducing and maintaining high threshold titres of biologically relevant **antibody** as well as persistence of active cytotoxic T cells recognizing multiple epitopes.

L27 ANSWER 59 OF 69 MEDLINE on STN

92309177. PubMed ID: 1613662. Infection of cynomolgus monkeys with a chimeric **HIV**-1/SIVmac virus that expresses the **HIV**-1 envelope glycoproteins. Li J; Lord C I; Haseltine W; Letvin N L; Sodroski J. (Department of Pathology, Harvard Medical School, Boston, Massachusetts. ) Journal of acquired immune deficiency syndromes, (1992) 5 (7) 639-46. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB Replication competent chimeric viruses that express the gag and pol proteins of SIVmac and the env proteins of **HIV**-1 were made. One such chimeric virus, SHIV-4, that expresses the vif, vpx, **vpr**, and nef regulatory genes of SIV and the tat and rev regulatory genes of **HIV**-1 replicated efficiently in cynomolgus monkeys. This model system can be used to evaluate the efficacy of anti-**HIV**-1 vaccines directed at the envelope glycoproteins, anti-**HIV**-1 envelope glycoprotein **antiserum** or monoclonal **antibodies**, and anti-**HIV**-1 drugs designed to inhibit the tat, rev, or env functions.

L27 ANSWER 60 OF 69 MEDLINE on STN

92239216. PubMed ID: 1571198. Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac). Luciw P A; Shaw K E; Unger R E; Planelles V; Stout M W; Lackner J E; Pratt-Lowe E; Leung N J; Banapour B; Marthas M L. (Department of Medical Pathology, University of California, Davis 95616. ) AIDS research and human retroviruses, (1992 Mar) 8 (3) 395-402. Ref: 74. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Simian immunodeficiency virus (SIV) is a designation for a group of related but unique lentiviruses identified in several primate species. A viral isolate from a rhesus macaque (i.e., SIVmac) causes a fatal AIDS-like disease in experimentally infected macaques, and several infectious molecular clones of this virus have been characterized. This report presents the complete nucleotide sequence of molecularly cloned SIVmac1A11, and comparisons are made with the sequence of molecularly

cloned SIVmac239. SIVmac1A11 has delayed replication kinetics in lymphoid cells but replicates as well as uncloned SIVmac in macrophage cultures. Macaques infected with virus from the SIVmac1A11 clone develop antiviral **antibodies**, but virus does not persist in peripheral blood mononuclear cells and no disease signs are observed. SIVmac239 infects lymphoid cells, shows restricted replication in cultured macrophages, and establishes a persistent infection in animals that leads to a fatal AIDS-like disease. Both viruses are about 98% homologous at the nucleotide sequence level. In SIVmac1A11, the **vpr** gene as well as the transmembrane domain of env are prematurely truncated, whereas the nef gene of SIVmac239 is prematurely truncated. Sequence differences are also noted in variable region 1 (V1) in the surface domain of the env gene. The potential implications of these and other sequence differences are discussed with respect to the phenotypes of both viruses. This animal model is critically important for investigating the roles of specific viral genes in viral/host interactions that cannot be studied in individuals infected with the **human immunodeficiency virus** (**HIV**).

L27 ANSWER 61 OF 69 MEDLINE on STN

92113582. PubMed ID: 1730943. Cells surviving infection by **human immunodeficiency virus** type 1: vif or vpu mutants produce non-infectious or markedly less cytopathic viruses. Kishi M; Nishino Y; Sumiya M; Ohki K; Kimura T; Goto T; Nakai M; Kakinuma M; Ikuta K. (Institute of Immunological Science, Hokkaido University, Sapporo, Japan. ) Journal of general virology, (1992 Jan) 73 ( Pt 1) 77-87. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Under conditions in which a clonal cell line (M10) isolated from a human T cell lymphotrophic virus type I-transformed MT-4 cell line was completely killed by infection with wild-type **human immunodeficiency virus** type 1 (**HIV**-1), equivalent M10 cells survived infection with **HIV**-1 vif, **vpr** or vpu mutant virus after transient cytopathic effects. Several cell clones, which were isolated from the proliferating M10 cells after infection with vif and vpu mutant viruses (M10/vif- and M10/vpu-), had heterogeneous **HIV**-1 phenotypes in terms of **HIV**-1 antigen expression, their syncytium forming capacity, reverse transcriptase activity and the infectivity of **HIV**-1 particles produced. When the replication kinetics of the **HIV**-1 particles produced were assayed in M10 cells, the clones could be classified into three types, i.e. type I producing non-infectious **HIV**-1, type II producing infectious **HIV**-1 with low replicative ability and type III producing infectious **HIV**-1 with a replicative ability similar to that of wild-type **HIV**-1. **HIV**-1 major viral cell proteins and virus particle fractions were almost typical in types II and III but not in type I. Electron microscopic examination of particles released by I, II and III clones revealed rare defective, predominantly defective and essentially normal virions, respectively. Northern and Southern blot analyses revealed no apparent deletion in the proviral DNA and mRNA prepared from these clones, except in the case of type I and II clones isolated from M10/vpu- which contained large deletions in the mRNAs for gag and gag-pol proteins. Thus, M10 cells surviving infection with **HIV**-1 vif or vpu mutants are heterogeneous, persistently expressing **HIV**-1 antigens and producing non-infectious or less cytopathic virus.

L27 ANSWER 62 OF 69 MEDLINE on STN

92024082. PubMed ID: 1926777. Analysis of alternatively spliced **human immunodeficiency virus** type-1 mRNA species, one of which encodes a novel tat-env fusion protein. Furtado M R; Balachandran R; Gupta P; Wolinsky S M. (Department of Medicine, Northwestern University Medical School, Chicago, Illinois 60611. ) Virology, (1991 Nov) 185 (1) 258-70. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB A polymerase chain reaction-based analysis was used to define the structures of the mRNAs that encode **human immunodeficiency virus** type-1 (**HIV**-1) regulatory and structural proteins in infected H9 cells. Twenty alternatively spliced mRNAs encoding the vif, **vpr**, env, nef, tat, and rev proteins were characterized. An evaluation of the coding

possibility of these subscripts recognized both leaky scanning and reinitiation at downstream initiation codons as mechanisms that may operate during translation of many of the polycistronic messages. Two new splice acceptor sites, one at nt 6018 defining a new mRNA coding for the env and vpu proteins and another at nt 8671 defining a novel tat-env fusion transcript, were characterized. The latter transcript expressed a novel protein p17tev that was immunoprecipitated by both polyclonal tat **antibodies** and monoclonals directed towards the C-terminal region of gp41. The p17tev protein was able to transactivate transcription from the **HIV**-1 LTR in transient transfection assays. The use of multiple alternative splice donor and acceptor sites and the generation of novel proteins may confer evolutionary advantages on the viral mutants encoding them and influence the course of clinical disease.

L27 ANSWER 63 OF 69 MEDLINE on STN

91143122. PubMed ID: 2149621. Identification and localization of **vpr** gene product of **human immunodeficiency virus** type 1. Sato A; Igarashi H; Adachi A; Hayami M. (Shionogi Institute for Medical Science, Osaka, Japan. ) Virus genes, (1990 Dec) 4 (4) 303-12. Journal code: 8803967. ISSN: 0920-8569. Pub. country: United States. Language: English.

AB The entire **vpr** gene of **human immunodeficiency virus** type 1 (**HIV**-1) was cloned into procaryotic and eucaryotic expression vectors. Production of authentic protein encoded by the gene in bacterial and mammalian cells was monitored by Western blotting using guinea pig **antisera** raised against an N-terminal 14-oligopeptide of the predicted **vpr** protein. A specific 12-kD protein was clearly detected with these **antisera**, but not with preimmune sera, in both cell systems, and this binding was blocked by the oligopeptide. These **antisera** also recognized a protein of the same size in several human T-cell lines infected with **HIV**-1. Western blotting analysis of subcellular fractions prepared from the cells producing wildtype **vpr** protein strongly suggested that the protein was membrane associated. A region within the **vpr** required for the stable expression of **vpr** product was also suggested by mutational analyses.

L27 ANSWER 64 OF 69 MEDLINE on STN

91122920. PubMed ID: 2149126. A synthetic protein corresponding to the entire **vpr** gene product from the **human immunodeficiency virus** **HIV**-1 is recognized by **antibodies** from **HIV**-infected patients. Gras-Masse H; Ameisen J C; Boutillon C; Gesquiere J C; Vian S; Neyrinck J L; Drobecq H; Capron A; Tartar A. (Biomolecular Chemistry Facility, CNRS-1309, Pasteur Institute, Lille, France. ) International journal of peptide and protein research, (1990 Sep) 36 (3) 219-26. Journal code: 0330420. ISSN: 0367-8377. Pub. country: Denmark. Language: English.

AB The 95 amino acid-protein encoded by the non-structural **vpr** gene of the **human immunodeficiency virus** type 1 (LAV-1BRU isolate) was chemically synthesized by solid phase methodology. The synthetic **vpr** protein was characterized by amino acid analysis, sequence analysis, RP-HPLC, and urea-SDS PAGE. Using a radioimmunoassay, **antibodies** to the synthetic protein were detected in sera of 25% of **HIV** 1-seropositive patients tested. Western blot analysis suggested that the **antibodies** preferentially recognize the dimeric form of **vpr**.

L27 ANSWER 65 OF 69 MEDLINE on STN

90257596. PubMed ID: 2341832. Speed of progression to AIDS and degree of **antibody** response to accessory gene products of **HIV**-1. Reiss P; Lange J M; de Ronde A; de Wolf F; Dekker J; Debouck C; Goudsmit J. (Department of Virology, University of Amsterdam, the Netherlands. ) Journal of medical virology, (1990 Mar) 30 (3) 163-8. Journal code: 7705876. ISSN: 0146-6615. Pub. country: United States. Language: English.

AB **Antibodies** to E. coli-produced **HIV**-1 nef, rev, tat, vpu, and **vpr** proteins were measured by enzyme immunoassay in serial sets of sera from 72 men seroconverting for **antibodies** to **HIV**-1 structural proteins, and from 190 initially symptom-free men who were seropositive for these **antibodies** at entry into the study. In the men seroconverting for **antibodies** to structural proteins the levels of nef-, rev-, and

**antibodies**, within 3 months of seroconversion, appeared to be lower in the five men progressing to AIDS, compared with the men remaining symptom-free during follow-up. Analysis of the prevalence of previously described **antibody** profiles to these accessory gene products was carried out. In all **HIV**-1 **antibody** seroconverters and in those **HIV**-1 **antibody** seropositive men with 15 or more months of follow-up who progressed to AIDS, there was a shift from predominantly nef- and vpu-specific **antibody** negative profiles in the men developing AIDS in the early years of the study to predominantly nef- and vpu-specific **antibody** positive profiles in men who developed AIDS later. Rev- and tat-specific **antibody** negative profiles were dominant in men progressing to AIDS throughout follow-up. No **vpr**-specific **antibody** profile occurred preferentially in the men progressing to AIDS throughout follow-up. Low **antibody** reactivity to accessory gene products nef, rev, and tat appears, like low anti-core **antibody** reactivity, to be associated with progression to AIDS relatively rapidly after infection with **HIV**-1.

L27 ANSWER 66 OF 69 MEDLINE on STN

90112005. PubMed ID: 2136912. **Antibody** response to viral proteins U (vpu) and R (**vpr**) in **HIV**-1-infected individuals. Reiss P; Lange J M; de Ronde A; de Wolf F; Dekker J; Danner S A; Debouck C; Goudsmit J. (Department of Virology, University of Amsterdam, The Netherlands. ) Journal of acquired immune deficiency syndromes, (1990) 3 (2) 115-22. Journal code: 8812597. ISSN: 0894-9255. Pub. country: United States. Language: English.

AB **Antibodies** to E. coli-produced **HIV**-1 **vpr** and vpu were determined by enzyme immunoassay in serial sets of sera from 72 men seroconverting for **antibodies** to **HIV**-1 structural proteins, and from 196 initially symptom-free men who were positive for such **antibodies** at study entry. First detection of **vpr**- and vpu-specific **antibodies** always was within 12 months of seroconversion for **antibodies** to structural proteins. In the combined cohort of 268 men, **vpr**- and vpu-specific **antibodies** were found persistently in 26 and 43% of men, respectively. **Vpr**- and vpu-specific **antibodies** were transiently detected in 3 and 7%, respectively, and intermittently detected in 18 and 13% of men, respectively. No association was found between the patterns of **vpr**- or vpu-specific **antibody** response and clinical outcome. In subjects with different patterns of **vpr**- and vpu-specific **antibody** response, no clear temporal relationship existed between the appearance or disappearance of **antibodies** and the onset of **HIV**-1-related disease.

L27 ANSWER 67 OF 69 MEDLINE on STN

90074393. PubMed ID: 2590553. Serum reactivity to **HIV**-1 accessory gene products distinguishes East African from West African **HIV** strains as infecting agent. Goudsmit J; Dekker J T; Boucher C A; Smit L; De Ronde A; Debouck C; Barin F. (Human Retrovirus Laboratory, Academic Medical Center, Amsterdam, The Netherlands. ) AIDS research and human retroviruses, (1989 Oct) 5 (5) 475-7. Journal code: 8709376. ISSN: 0889-2229. Report No.: PIP-060605; POP-00193107. Pub. country: United States. Language: English.

AB The existence of dual infections with **human immunodeficiency virus** (**HIV**)-1 and 2 in West African countries has been controversial, although the current consensus is that dual infection is not the cause of the extensive cross-reactivity observed between these 2 viruses. To evaluate the role of **antibody** reactivity to the **HIV**-1 accessory gene products in type-specific **HIV** serology, proteins encoded for nef, tat, rev, **vpr**, and vpu were developed and used as an antigen. 5 of the 7 exclusively **HIV**-2 reactive sera were not reactive to the **HIV**-1 accessory gene products. Moreover, the 2 sera that showed reactivity to the **HIV**-1 envelope were the only ones reactive to **HIV**-1 accessory gene products. These findings indicate that type 2 viruses may be as diverse as type 1 viruses. A subsequent analysis of sera from 24 West Africans revealed reactivity with a simian immunodeficiency virus (SIV) peptide but not with an **HIV**-1 peptide previously shown to be discriminatory in a

assay between **HIV**-1 and **HIV**-2. Compared to 25 control sera from East Africans, the West Africa sera had significantly lower reactivity to **antibodies** specific to nef, tat, and rev; there was not reactivity to **vpr** and vpu. 38% of the West African sera compared with 93% of the East African sera showed reactivity to **HIV**-1 accessory gene products. It is concluded that, while reactivity to the **HIV**-1 accessory gene products **vpr** and vpu indicate **HIV**-1 infection, reactivity to the other accessory gene products cannot be used to identify virus type given the documented cross-reactivity to **HIV**-1 accessory gene products of **antibodies** elicited by **HIV**-2 strains.

L27 ANSWER 68 OF 69 MEDLINE on STN

89259383. PubMed ID: 2724559. Isolation of **HIV**-2 from AIDS patient in Ghana and analysis of sero-reactive patterns of Ghanaian sera. Hayami M. Nippon rinsho. Japanese journal of clinical medicine, (1989 Jan) 47 (1) 141-8. Journal code: 0420546. ISSN: 0047-1852.
Report No.: PIP-061100; POP-00203853. Pub. country: Japan. Language: Japanese.

AB Institute for Virus Research, Kyoto University, and Noguchi Memorial Research Institute in Ghana jointly isolated **HIV**-2 from a Ghanaian AIDS patient. Ghanaian **HIV**-2[GH-1] was similar in genomic organization to, but different in Restriction Enzyme Maps from the first isolated case, French LAV-2 (**HIV**-2ROD). This fact is significant in suggesting multiplicity of **HIV**-2 virus. **HIV**-2[GH-1] was different from LAV-2 in its antigenicity of envProtein. Sero-reactive patterns of 125 Ghanaians including 57 AIDS/ARC patients were analyzed using **HIV**-1, **HIV**-2, and SIVAGM as antigen. 4 groups were recognized. Group I was **HIV**-1 only positive; Group II, **HIV**-2-only positive; Group III, mixed **HIV**-1 and **HIV**-2 positive; Group IV reacted to gag protein of **HIV**-1, **HIV**-2 and SIVAGM but not to envProtein of any. 24 Ghanians were **HIV**-1 positive; 38 were **HIV**-2 positive. 19 out of 20 **HIV**-2-only positive patients were AIDS/ARC patients. Symptoms and route of infection from **HIV**-1 and **HIV**-2 AIDS seem to be similar but **HIV**-2 is weaker pathologically and in communicability. **HIV** positive patients were mostly found in the big city such as Accra among young women, especially prostitutes who were migratory "workers" in neighboring countries along the Ivory Coast. Sero-reactive patterns of Ghanaians with SIV as antigen had been known for some time to be different from those of Kenyans in the east coast and those of Gabonese. While KEnyan's **antibody** was high for **HIV**-1 and lower for SIVAGM, Ghanian's was higher for SIVAGM than **HIV**-1. Gabonese's was in between. With discovery of LAV-2 (**HIV**-2ROD) and Ghanian hybridization with its DNA probe Ghanaian's was found to have among other things VPX and **VPR** in common with **HIV**-2ROD.

L27 ANSWER 69 OF 69 MEDLINE on STN

89089998. PubMed ID: 2850118. Animal models for **HIV** infection and AIDS: memorandum from a WHO meeting. Anonymous. Bulletin of the World Health Organization, (1988) 66 (5) 561-74. Journal code: 7507052. ISSN: 0042-9686.
Report No.: PIP-054632; POP-00186454. Pub. country: Switzerland. Language: English.

AB The **human immunodeficiency virus** is a member of the lentivirus subfamily of the retrovirus family. Retroviruses are RNA viruses which code for an RNA-dependent DNA polymerase (reverse transcriptase), which transcribes the RNA genome into a DNA provirus which, on integration with the host DNA, directs the synthesis of new virions. The RNA genome consists of a gag gene, which codes for the viral core proteins, a pol gene, which codes for the reverse transcriptase, an env gene, which codes for the glycoproteins of the viral envelope, and several genes (tat, rev, vif, **vpr**, and nef), that code for regulatory proteins. At each end of the genome are long terminal repeats, that contain regulatory elements for transcription. There are 3 subfamilies of Retroviridae (Oncovirinae, Spumavirinae, and Lentiverinae). The Lentiverinae ("slow viruses") include the bovine immunodeficiency virus (BIV), the feline immunodeficiency virus (FIV), the human immunodeficiency viruses (**HIV**), and the simian immunodeficiency viruses (SIV). SIV has been isolated from

macaques (mac), African green monkeys (agm), sooty mangabeys (sm), and mandrills (mnd). Only SIVmac causes an AIDS-like disease in its natural host, but it is genetically closer to **HIV**-2 than to **HIV**-1. SIVsm causes an AIDS-like disease in macaques, but not in the sooty mangabey. Monkeys infected with SIV develop diarrhea, wasting, decrease in T4 lymphocytes, lymphadenopathy, development of giant cells, and encephalitis, as well as opportunistic infections. Kaposi's sarcoma, however, has not been found in SIV-infected primates. Virus is recovered from peripheral blood mononuclear cells and the brain. SIV models are useful for understanding the natural history of primate lentiviruses, for defining the pathogenesis of AIDS, and for developing vaccines. The ideal model would be one in which **HIV** causes AIDS, but so far only chimpanzees and gibbons have successfully been infected with **HIV**-1, and although virus, is recovered from peripheral blood mononuclear cells of chimpanzees within 2 weeks of infection, and 2 animals have lost **antibodies** to the p24 protein, none has so far developed clinical AIDS. Attempts to develop vaccines to immunize chimpanzees are continuing. Nonprimate lentiviruses include the visna virus, the feline immunodeficiency virus, and the bovine immunodeficiency virus. The visna virus infects fibroblasts by fusion of the viral envelope with the plasma membrane of the fibroblast; it infects macrophages by endocytosis. Infected macrophages regulate the production and dissemination of viral particles. The feline immunodeficiency virus infects T-lymphocytes of cats and produces oral, gastrointestinal and respiratory pathology as well as lymphadenopathy and opportunistic infections. Bovine immunodeficiency-like virus causes a generalized lymphadenopathy similar to that seen in AIDS-related complex.

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FILE 'USPATFULL' ENTERED AT 07:45:15 ON 28 JUN 2004
E WEINER DAVID/IN
L1 40 S E5
L2 49 S E3 OR E5
L3 26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4 26 S L3 AND ANTIBOD?
E LEVY DAVID/IN
L5 23 S E3
L6 23 S L5 NOT L4
E REFAELI YOSEF/IN
L7 6 S E3
L8 0 S L7 NOT (L5 OR L1)
E MONTAGNIER LUC/IN
L9 99 S E3
L10 9 S L9 AND (VPR OR VIRAL PROTEIN R)
L11 33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L12 594 S L11 AND (VPR OR VIRAL PROTEIN R)
L13 548 S L12 AND ANTIBOD?
L14 156 S L13 AND (ANTIBOD?/CLM)
L15 35 S L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)
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L17 9 S E3 OR E4
L18 2 S L17 AND (VPR OR VIRAL PROTEIN R)

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L20 121 S L19 AND (VPR OR VIRAL PROTEIN R)
L21 44 S L20 AND ANTIBOD?

FILE 'MEDLINE' ENTERED AT 08:19:47 ON 28 JUN 2004
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L26          623 S L25 AND (VPR OR VIRAL PROTEIN R)
L27           69 S L26 AND (ANTIBOD? OR ANTISER?)

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L1             40 S E5
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L3             26 S L2 AND (VPR OR VIRAL PROTEIN R)
L4             26 S L3 AND ANTIBOD?
                  E LEVY DAVID/IN
L5             23 S E3
L6             23 S L5 NOT L4
                  E REFAELI YOSEF/IN
L7              6 S E3
L8              0 S L7 NOT (L5 OR L1)
                  E MONTAGNIER LUC/IN
L9             99 S E3
L10             9 S L9 AND (VPR OR VIRAL PROTEIN R)
L11         33171 S (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
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L14           156 S L13 AND (ANTIBOD?/CLM)
L15            35 S L14 AND (VPR/CLM OR VIRAL PROTEIN/CLM)
L16            26 S L15 NOT (L1 OR L9)
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L21            44 S L20 AND ANTIBOD?

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L22           265 S E3 OR E1
L23            17 S L22 AND (VPR OR VIRAL PROTEIN R)
L24             3 S L23 AND ANTIBOD?
L25       143517 S (HIV OR HTLV-III OR HUMAN IMMUNODEFICIENCY VIRUS OR HUMAN T-C
L26          623 S L25 AND (VPR OR VIRAL PROTEIN R)
L27           69 S L26 AND (ANTIBOD? OR ANTISER?)

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